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16. ABSTRACT

This book contains the proceedings of a symposium entitled "Behavioral Toxicology: An Emerging Discipline", held in conjunction with the Southwest Psychological Association meetings, April 30, 1976 in Albuquerque, New Mexico. Authors of formal presentations later reviewed and enlarged their contributions.

The participants were drawn from a number of different disciplines including toxicology, pharmacology, psychology, physiology, and veterinary medicine.

The intent of the book is to provide a "state of the art" overview of the area of behavioral toxicology. It includes chapters discussing some of the most fundamental aspects such as sources of funding (Chapter 1), and basic methodological and experimental considerations (Chapters 2, 3, 4). In addition, there are chapters that address specific areas of investigation including the utilization of techniques to assess the interaction of the animal with its environment (Chapters 5, 6, 7), considerations in sensory toxicology (Chapter 8), and employment of electrophysiological techniques (Chapters 9, 10).

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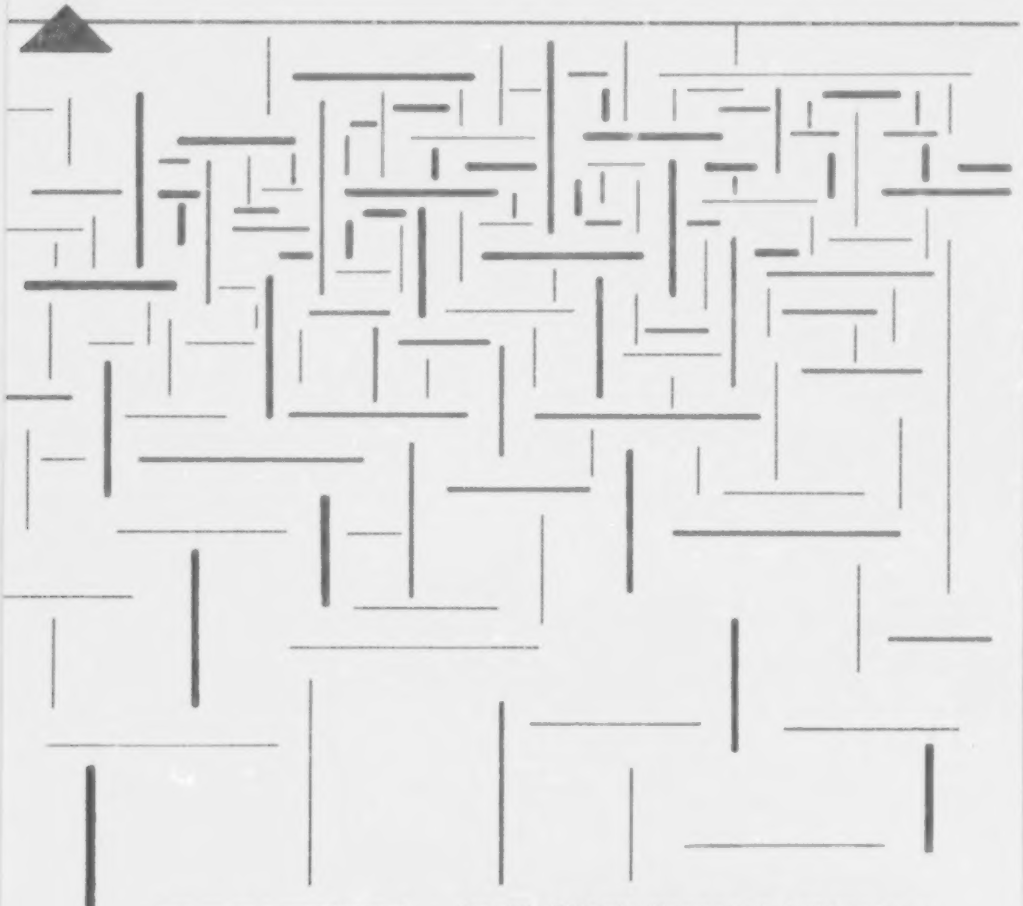

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BEHAVIORAL TOXICOLOGY: AN EMERGING DISCIPLINE

Proceedings of the Southwest Psychological Association Annual Meeting
April 30, 1976
Albuquerque, New Mexico

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FOREWORD

Cerebration is among those characteristics that most decisively distinguish humans from other animals. The study of higher thought processes has intrigued researchers for centuries, but conceptual, as well as methodological problems, have made progress frustratingly slow. Recent years, however, have seen a variety of breakthroughs in areas such as neurochemistry as well as the application of information theory to artificial intelligence. There has also emerged a growing concern for the application of behavioral research to societal problems. It seems natural then, that one of our recent, most pressing societal problems—the reconciliation of man's interaction with his environment—has become an important driving force in neurobehavioral research. In effect, the title of this symposium, "Behavioral Toxicology: An Emerging Discipline" underscores the somewhat embryonic nature of this area of research.

Much environmental toxicology (appropriately) focuses on relatively gross changes in biochemistry, morphology, or physiology. It is equally appropriate, however, for the behavioral toxicologists to concern themselves with the subtle alterations environmental agents may exert on man's capacity to cope with his...environment.

Environmental health effects research has many inherent difficulties. Behavioral research has many inherent difficulties. The juxtaposition of these two disciplines results inescapably in a multiplying effect of the research problems encountered. I have encouraged and supported my colleagues in environmental neurobehavioral research over the years—but from a distance. Thus, it is personally gratifying to me to be able to recognize in a more explicit fashion the progress they are making. This symposium represents one more important step forward in the consolidation, coordination and promotion of the emerging discipline of behavioral toxicology.

John H. Knelson, M.D.
Director
Health Effects Research Laboratory
Research Triangle Park, N.C. 27711

PREFACE

This book contains the proceedings of a symposium entitled "Behavioral Toxicology: An Emerging Discipline" held in conjunction with the Southwest Psychological Association meetings, April 30, 1976, in Albuquerque, New Mexico. Authors of formal presentations later reviewed and enlarged their contributions. In emphasizing the interdisciplinary nature of this area, the participants were drawn from a number of different disciplines including toxicology, pharmacology, psychology, physiology, and veterinary medicine. The audience that this publication addresses is not only contemporaries in the field but also those researchers in behavioral toxicology. Thus the intent of this book is to provide a "state of the art" overview of the area. We have attempted to do this by incorporating chapters that discuss some of the most fundamental aspects such as sources of funding (Chapter 1), and basic methodological and experimental considerations (Chapters 2, 3, 4). In addition, there are chapters that address specific areas of investigation including the utilization of techniques to assess the interaction of the animal with its environment (Chapters 5, 6, 7), considerations ins sensory toxicology (Chapter 8), and employment of electrophysiological techniques (Chapters 9, 10).

CONTENTS

1. *John A. Santolucito*
   Overview of Federal Funding Avenues for Support of Neurotoxicology

2. *W. B. Buck, D. L. Hopper, W. L. Cunningham, and G. G. Karas*
   Current Experimental Considerations and Future Perspectives in Behavioral Toxicology

3. *Harold Zenick*
   A Review of the Developmental Models Employed in Behavioral Toxicology

4. *Robert L. Bornschein and I. Arthur Michaelson*
   Methodological Problems Associated with the Exposure of Neonatal Rodents to Lead

5. *Stata Norton*
   Observational Techniques in Behavioral Toxicology

6. *Lawrence Reiter, George Anderson, Miriam Ash, and L. Earl Gray*
   Locomotor Activity Measurements in Behavioral Toxicology:Effects of Lead Administration on Residential Maze Behavior

7. *Phyllis Mullenix*
   Altered Behavioral Patterning in Rats Postnatally Exposed to Lead: The Use of Time-Lapse Photographic Analysis

8. *Hugh L. Evans*
   Behavioral Assessment in Sensory Toxicology

9. *Dorothy E. Woolley*
   Electrophysiological Techniques in Toxicology

10. *David Otto*
    Neurobehavioral Toxicology: Problems and Methods in Human Research

1. OVERVIEW OF FEDERAL FUNDING AVENUES FOR SUPPORT OF NEUROTOXICOLOGY

JOHN A. SANTOLUCITO

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Although this symposium is entitled "Behavioral Toxicology," I believe it is acceptable, if not desirable, for me to address the general subject of neurotoxicology. Simply defined, neurotoxicology is the systematic study of unwanted effects of toxic agents on the nervous system. Recent interest in neurotoxicology is largely due to the growing concern over the number of toxic agents to which we are exposed in our highly industrialized society. The number of synthetic materials being produced, as well as the large amounts of industrial and municipal waste products being introduced into the environment, have all added significantly to the urgency of neurotoxicologic research.

In order to understand adequately the biological effects of environmental insults, it is necessary to consider the potential of the insult to impair functioning of the nervous system by direct or indirect alteration of neuronal physico-chemical states. Such impairment may appear, for example, as changes in the mental ability of an individual to react, remember, or learn; as changes in emotional states, such as anxiety or depression; or as changes in neuroendocrine function. These changes may result in decreased capability to cope with everyday living conditions, with a subsequent reduction in the "quality" of life. Health effects such as these, which fall short of morbidity and mortality, become increasingly important as the requirements for our society become more complex and demanding.

It is not necessary to stress further the importance of neurotoxicology in the overall assessment of responses to toxicologic agents. However, as a discipline, it is in a relatively early developmental stage, having received little formal recognition apart from that given to specific occupational risks prior to 1969 (Weiss and Laties, 1969).

The recent establishment of neurotoxicology as a discipline is attracting the interest of a new cadre of investigators seeking assurance that the Federal Government is interested in and supportive of the discipline. A review of inhouse research and extramural support for the past decade provides this assurance. All Federal agencies having responsibility in the field of toxicology have recognized neurotoxicology as an important investigatory discipline since the middle 1960's. While the interest initially focused on the direct effects of toxic agents on the central nervous system (CNS), it later encompassed the use of behavioral and/or neurophysiologic indicators of toxicity in which the CNS may not be the primary target (Colleti and Krueger, 1970). The present diversity of interest includes physical contaminants (e.g., noise and microwave) as well as chemical contaminants in the environment and psychologic as well as physiologic effects (Berglund *et al.*, 1974).

The problem faced by Federal agencies in evaluating the neurotoxicologic effects of numerous insults, given that the CNS integrates and is reactive to the status of the whole organism, is the lack of definitive equations relating alterations in neurophysiologic indices, including behavior, to life span, health, productivity, or well-being. For this reason, available funds for support of neurotoxicologic research are modest despite the recognition of its importance. It is also worth noting that in times of a tighter economy many agencies tend to shift extramural support disproportionately in favor of contracts over grants. That is to say, agencies use available funds to solve specific problems rather than to expand a general information base having no immediate payoff. This tendency should cause no great concern to those seeking research support. It simply necessitates formulating specific objectives which meet the specific research needs of the agencies. Grant monies are not unavailable. Rather, they may be relatively more difficult to obtain.

Contracts may be competitive or noncompetitive, solicited or unsolicited. Federal Procurement Regulation states, "All purchases and contracts shall be made on a competive basis to the maximum possible extent." Because individual agencies' needs will vary, so will their interpretation of maximum possible extent. All solicited competitive contracts are initiated by an agency in the form of a request for proposal (RFP) published in the *Commerce Business Daily** along with a closing date by which proposals must be received and the name and address of the requesting agency. Unsolicited contracts are considered when they represent original effort by the offeror in the form of new and unique ideas.

It is therefore apparent that the research needs of an agency and the research capabilities of an investigator must be brought together. Research needs and information about funding mechanisms can be obtained from each agency's office or division responsible for extramural research or grants and contracts. For example, a publication entitled "How to Do Business With EPA: A Guide to Requirements for Grants and Contracts," is available from Environews, Inc.,

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*Available from the Superintendent of Documents, Government Printing Office, Wash., D. C. 20402.

This symposium discusses some of the testing methodologies currently used in neurotoxicology, including behavioral, neurochemical, and electrophysiologic techniques. One of the primary goals of neurotoxicology is to provide data for use in the evaluation of potential health hazards associated with exposure to various environmental pollutants. A thorough understanding of the methods employed and of the properties being measured is critical to achieving this objective. Sources of variability, control measures, and validation procedures associated with the testing methodologies must be known. Baseline performance or response of the test animal used and confirmation of the composition or purity of the toxic agent being tested are equally important. Obviously, the more reliable and reproducible the test system, the more we understand the biological effects of toxins and the better equipped we are to define the risks involved in exposure. Quality assurance in biological research is a matter of prime concern to Federal agencies.

Neurotoxicology is a young and vigorous discipline. While in its infancy, it is being challenged to provide answers to complex and multifaceted real-world problems. This symposium was convened to address some of these problems and to assess current progress in several areas of neurotoxicology related to environmental pollutants. The planning and implementation of this symposium, the contents of its timely papers, and the expertise in attendance afford confidence that neurotoxicology will meet these challenges.

ADDENDUM

I. National Institutes of Health
Office of Extramural Research Support
Bethesda, Maryland 20014

Some of the Institutes of NIH will be more likely to have interest in neurotoxicology than others. For example: National Institute of Environmental Health Sciences; National Institute of Mental Health; National Institute of Child Health and Human Development. If it seems probable that a research capability would be of interest to one of the Institutes in particular, it would be advisable to so indicate.

II. National Institute of Occupational Safety and Health
Office of Extramural Research Support
5600 Fishers Lane
Rockville, Maryland 20852

III. Environmental Protection Agency

In EPA there are two Health Effects Research Laboratories with interest in behavioral toxicology. They receive unsolicited proposals through the Grants Administration Division, Environmental Protection Agency, Washington, D.C. 20460.

IV. Food and Drug Administration

Each of the units receives proposals directly as follows:

For environmental pollutants
Assistant Director for
Scientific Coordination
National Center for Toxicological
Research
Jefferson, Arkansas 72079

For food additives/contaminants
Director, Bureau of Foods
200 C St., S.W.
Washington, D. C. 20204

For therapeutic drugs
Director, Bureau of Drugs
200 C St., S.W.
Washington, D. C. 20204

For diagnostic agents
Director, Bureau of Biologics
200 C St., S.W.
Washington, D. C. 20204

For veterinary products
Director, Bureau of
Veterinary Medicine
200 C St., S.W.
Washington, D. C. 20204

V. National Aeronautics and Space Administration
Director, Office of University Affairs (Code P)

or

Director, Office of Life Sciences (Code SB)
NASA Headquarters
Washington, D. C. 20547

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Berglund, B., Berglund, U., and Lindvall, T. (1974). Scaling of annoyance in epidemiologic studies. In *Recent Advances in the Assessment of the Health Effects of Environmental Pollution: International Symposium Proceedings.* Commission of the European Communities, Luxembourg.

Colleti, R. B. and Krueger, K. K. (1970). *Proceedings of the Workshop on Behavioral Assays in Uremia.* U.S. DHEW Publication No. (NIH) 72-37.

Weiss, B. and Laties, V. G. (1969). Behavioral pharmacology and toxicology. *Annu. Rev. Pharmacol.* 9, 297-326.

2. CURRENT EXPERIMENTAL CONSIDERATIONS AND FUTURE PERSPECTIVES IN BEHAVIORAL TOXICOLOGY

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G. G. KARAS

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Veterinary Diagnostic Laboratory
College of Veterinary Medicine
Iowa State University

Introductory Comments

Any discussion of behavioral toxicology generally begins with an attempt to put into words a concept which, as yet, has not been fully developed. This "definition" problem manifests itself as an "identity" problem, not only among ourselves but also in the eyes of our colleagues in other disciplines. The urge to define comes from a feeling that we are well past puberty and represent a mature field of endeavor. However, considering that our evolutionary process has only recently begun (we did not actually agree on what to call ourselves until the late 1960's), a final definition may be premature.

Rather than attempt a definition, we propose to discuss the scope and multidisciplinary aspects of behavioral toxicology. Whether one considers behavioral toxicology as another approach for the study of the effects of toxicants on an organism or as a means of studying animal and human behavior as affected by neurotoxicants, we can surely agree that it is a field of endeavor that requires the expertise of many disciplines.

It is unlikely that a single researcher can be found who possesses the many and varied skills needed for the conduct of behavioral toxicology studies. Therefore, studies using the behavioral toxicologic approach usually involve a multidisciplinary team effort. The various disciplines that are represented on this team will be determined by the availability of individuals with a specific expertise and the objectives of the research. Listed below are some of the disciplines that may be found in a behavioral toxicology research program:

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*This work was supported by Environmental Protection Agency Contract 68-02-2288 to Dr. William B. Buck, Director, Behavioral Toxicology Laboratory, Iowa State University, Ames, Iowa 50010.

1. psychology
2. toxicology
3. veterinary and/or human medicine
4. chemistry
5. physiology
6. pathology
7. biophysics
8. biomedical engineering
9. computer science
10. statistics

The behavioral toxicologist strives to devise sensitive techniques to evaluate the possible consequences of exposure to new and untested chemicals as measured by the total integrated function of the organism being tested. Those toxicants having direct effects on the nervous system, whether they be sensory, motor, or cognitive, are more likely to be studied in behavioral toxicology. However, toxicants which have their primary effect on other organ systems, such as the liver and kidney, may also be sensitively assessed using the multidisciplinary behavioral toxicologic approach. Perhaps one can summarize the goals of the behavioral toxicologist as: (1) the utilization of behavior for the assessment of the effects of toxicants, (2) the correlation of behavioral changes with concomitant physiopathologic alterations (overt clinical signs, pathologic changes, electrophysiologic and biochemical alteration, and death), and (3) the assessment of the effects of toxicants on behavior (sensory, motor, and cognitive).

Behavior as an Index of Toxic Effects

One need only make a cursory review of the literature over the past 10 years or scan the references of some of the more recent behavioral toxicology review papers (Weiss and Laties, 1975; Bignami, 1976) to appreciate the amount of scientific research that has accepted behavior as a viable index for understanding the effects of toxic substances. The increased use of behavior should give gratification to those early researchers who felt that this was a missing dimension in experimental environmental toxicology (Ruffin, 1963; Brimblecombe, 1968).

A majority of behavioral toxicologic research has dealt, for the most part, specifically and directly with behaviorally measuring the effects of exposure to toxic compounds. This "direct effect" type of research (the examination of the effects on behavior only) has been and remains important for the following reasons. First, it has helped in understanding the kind and the magnitude of behavioral effects that may result from exposure to toxic substances, effects that would not be apparent from an examination of only neuroenzyme or structural changes. Investigations from our laboratory over the past 10 years which have involved the study of the effects of exposure to lead (Van Gelder, *et al.*, 1973; Carson, *et al.*, 1973; Carson, *et al.*, 1974), chlorinated hydrocarbons (Sandler, *et al.*, 1969; Smith, *et al.*, 1976) and organophosphates (Reischl, Van Gelder, and Karas, 1975; Van Gelder, 1975), provide examples of this type of research. Within the confines of these experiments, we have been able to examine the behavioral functioning of an organism as it has been affected by a specific toxicant.

Second, this type of investigation has helped to provide a research foundation which gives the discipline credibility. By demonstrating that behavior can be affected by toxic substances and that it can be as sensitive as other toxicologic indices, we have established not only the credibility of behavior as a measurement tool, but also the viability and reliability of the discipline as a competent information provider.

However, it will probably be through an "indirect" research approach that behavior will have its greatest utility and value as a research tool. That is, when we examine behavior in conjunction with, rather than in lieu of other toxicologic methods, we will be moving towards a better understanding of the effects of toxic compounds. That is not to lessen our roles either as behaviorists or toxicologists. Just as one cannot hope to get a complete image of the effects of a toxic substance or the overall functioning of an organism by looking at enzyme levels, tissue levels, or an EEG alone, similarly, one cannot hope to get a complete picture by looking solely at behavior. The multidisciplinary approach, by combining people and ideas from different disciplines, encourages attempts at correlating behavior with other indices of toxicosis in order to obtain a more complete picture of the *total* functioning of an organism. The behavioral toxicologist, or behavioral toxicology research team, then, has an important place in terms of the overall understanding of environmental toxicology.

Clinical Observations as a Source of Research Hypotheses

Clinical observations are perhaps the most direct and the oldest source of information available to the toxicologist concerning the effects of a toxic agent on an organism. Such observations can provide not only valuable information as to the source and treatment of a toxicosis, but also important clues in the continuing effort to delineate toxic effects, and they are, therefore, important to the formulation of viable research hypotheses.

One of the most apparent and readily determined signs of illness of toxicosis is a change in the reflexive responses of the organism. Alterations in eye blink, startle response, clasping, righting, or withdrawal, for example, give good indications as to the source, extent, and severity of toxicoses. Knowledge of reflexive response changes can aid in isolating toxic effects to various body systems (CNS, vasculature, sensory organs, motor system, and musculature).

Many toxic agents have effects which are not associated with overt clinical signs of which are manifested in situations or in organisms that do not lend themselves to controlled experimentation. In such situations, the effects of toxic agents on naturally occuring responses (although of interest in their own right) can be a particularly useful resource for research hypotheses. By "natural responses" we mean behavioral outputs characteristic of an organism which require no experimenter training and which occur with a minimum of learning or experience on the part of the organism. Examples of such natural responses include herding behavior, many of the stereotypic mating responses, characteristic social behaviors, dominance hierarchies, orientation (homing, spawning), and territorial behaviors. All of these behaviors are readily observable and subject to toxicologic insult. The potential behavioral hypotheses are innumerable.

Finally, a distinction must be made between clinical "signs" and "symptoms." Clinical signs, such as changes in blood pressure or respiration, may appear with a toxicosis in both humans and animals. However, symptoms are available to the toxicologist only from human subjects and are merely the subjective report given to the toxicologist by the subject. By extrapolation, symptoms reported by human subjects may provide important clues to toxicoses and their effects in animals; whereas, the reverse is obviously not available as a resource for research hypotheses from animals.

Research Paradigms and Measures

Much of the research in behavioral toxicology has been paradigm oriented, resulting directly from an effort to find behavioral paradigms which are both sensitive to toxicologic insult and able to provide answers to questions of specific behavioral interest.

Activity Tests

A favorite measurement of the psychopharmacologist and behavioral toxicologist has been the general activity of the organism. The term "activity" has been most often used in reference to general motor activity. The method used to obtain the measure is important in the use of activity measures. General activity can be measured in revolving wheels, stabilimeters, or tilt cages, by photoelectric means, by ultrasonic or resonant circuits, or by direct observation. Each of these methods of obtaining activity levels is subject to a number of variables and may be differentially affected by each. The interpretation of treatment effects due to a toxic insult can be most meaningfully understood if stable pretreatment baselines are established, if the animal is properly habituated to the activity apparatus, and if extraneous variables such as handling, light, noise, health, age, sex, motivation, diet, population density, strain, and species are controlled. For discussion of this topic see Finger (1972).

Although many activity apparatuses have the advantage of simplicity, they cannot provide the wealth of information available through direct observation. This method, when used properly by taking precautions against investigator bias, avoiding subject-experimenter interaction, and insuring standards of behavioral classification, can provide activity information directly relevant to a number of potential research hypotheses. This is not possible with activity measuring devices. Norton (1973) has recently introduced a procedure, which has subsequently been modified and automated in our laboratory, whereby some of the sources of error and difficulties associated with employing the method of direct observation can be reduced. This method of activity determination is called video pattern recognition. It employs a computer algorithm to determine the occurrence of experimenter-defined behavioral acts. Observation is via a movie camera input to a computer system. This methodology has great potential both in objectifying a classically used observation method and in providing a means of carefully examining aspects of a variety of behavioral activities which have been previously difficult or impossible to obtain. This method is described in greater detail by Norton in Chapter 5 of this study, while application of a macroanalytic technique is discussed by Reiter (Chapter 6) and microsampling by Mullenix (Chapter 7).

Developmental Testing

Evidence that the immature organism is more likely to be affected by a number of toxic agents is fairly well accepted among toxicologists. This research has established that changes seen in an organism may be specific to one period of the life span, thus making the time of exposure a critical variable in delineating a toxic effect. In Chapter 3, Zenick reviews various developmental models that have been employed in behavioral toxicology. The nature of a toxic insult which occurs at one or more periods in the development of the organism and manifests itself in another can best be detected by the use of a longitudinal experimental design. The long-term assessment of the effects of toxicants on animals or humans has only recently begun to be exploited, and we need a great deal of research in this area if we are to understand the interaction of the developmental processes with other variables of interest to the behavioral toxicologist. A recent example of this research approach is discussed by Spyker (1975).

Appetitive and Aversive Conditioning

Behavioral toxicologists have employed a broad range of classical, instrumental, and operant paradigms in their efforts to elucidate the effects of toxic agents on behavior. It is beyond the scope of this paper to discuss the nuances of the many paradigms which have been employed; however, we feel that some comments abouts the selection and use of behavioral paradigms are worthwhile. Of primary importance to the selection of a behavioral paradigm are the realizations that behavior is not a single, simple entity and that failure to find a toxicologic effect on behavior as measured under one set of circumstances with one paradigm does not preclude effects with other paradigms under similar or even dissimilar circumstances. The rule of behavior is its complexity and variability; it is a function of antecedent conditions, current stimulus circumstances, the properties of the behavior act itself, and the consequences of the act.

The constraints on the selection of a behavioral paradigm are many: the hypotheses to be tested, the subjects utilized, the effects sought (whether chronic or acute), the nature of the toxicant, and the availability of equipment and funds are only a few of the important factors in the selection of a paradigm. The best tool available to the toxicologist to aid in the selection process is a thorough evaluation of the research literature. It is hard to imagine a greater frustration than discovering that the paradigm one has selected and in which one has invested many hours and thousands of dollars has been shown by colleagues to be inappropriate on the basis of a number of criteria.

We have found in our laboratory that when studying the chronic effects of various toxicants, it is strongly advisable to begin the experiment with as many subjects as reasonably possible. For example, the need for a large number of subjects is particularly true for between-group designs employing highly toxic agents with potentially cumulative effects. A final admonition in the selection and utilization of a behavioral paradigm is to expect the unexpected. Valuable insights, or even factors in the delineation of a toxic effect, can be lost by a mind that has an *a priori* set of expectations which precludes an insightful examination of the data obtained from any paradigm which might be utilized.

Sensory Processes

The sensory systems are the means by which our information-processing brains interact with the environment, and as such these systems place important functional constraints on behavior. The study of sensory systems is one of the oldest research areas in experimental psychology, and the methods employed in this study are well developed and have stood the test of time. We refer of course to the traditional psychophysical methods and their contemporary improvements. By utilizing the method of limits, the method of average error, or the method of constant stimulus differences, we can determine important information concerning the effects of a toxicant on the absolute threshold, terminal threshold, difference thresholds, and the discriminability of stimuli.

More frequently than is desirable, behavioral tests are conducted with complete disregard for the effects of toxicants on important sensory systems. Deficiencies ascribed to disruptions in short-term retention in a visual delayed response task could well occur because of an alteration in the visual system. We can apply this same logic to a number of the behavioral paradigms we have employed in assessing toxic effects. Recent efforts in our laboratory (Reischl *et al.*, 1975) and by others (Hanson, 1975) have shown significant alterations in sensory systems due to toxic insult. In Chapter 8, Evans examines the behavioral principles likely to aid in the precise definition of sensory impairment caused by toxins.

Cognitive Processes

In the last decade, there has been a great expansion of research on cognitive processes in humans and, more recently (the past five years), with nonhuman primates. The growth of cognitive research, particularly with nonhuman primates in the areas of short- and long-term retention, forgetting, and language acquisition, can provide the toxicologist with promising behavioral tools. With the upsurge of research on human cognitive processes, there has been a corresponding development in cognitive theory. We feel that within this area the behavioral toxicologist might find unifying concepts relating findings with animal subjects to those with humans. It has been our experience that the more complex testing paradigms have been more sensitive to toxicologic insult, particularly during the period of acquisition. Of special interest is the fact that some cognitive processes appear to be fairly localized functionally within the CNS, for example, short-term memory and the hippocampus, language and speech centers in the left cortical hemisphere, and the prefrontal cortex and delayed response deficit.

We are hopeful that the behavioral toxicologist will utilize the research paradigms available to study cognitive processes in both humans and animals so as to delineate subtle behavioral changes resulting from long-term chronic exposures to a number of central nervous system toxicants.

Variables of Critical Interest

Interacting with all of the experimental approaches which we have just mentioned are a number of specific variables, which, if not controllable or manipulable in some way, must at least be recognizable as contributing their effects to our data.

One of these variables could be simply the species used within a given experiment. One should always assume that his data is species specific until interspecies experiments prove otherwise. Furthermore, one could have intraspecies effects, such as one strain of experimental subjects displaying behaviors which are slightly different from another strain of subjects comparably exposed to a given chemical. In either case, the species and strain issues raise a question about extrapolation of our research data.

A second variable which has shown itself to be of considerable interest is the dose-response relationship. High-dose, acute-exposure experiments have considerable importance in that they suggest tasks which might have potential for being sensitive to much lower chronic exposure levels. For example, early in our experimental program, we found that the conditioned avoidance response task did not appear to be sensitive to dieldrin exposure in sheep even at levels that were sufficient to produce convulsions and other overt clinical signs of toxicosis. Most behavioral toxicologic research, however, will probably deal in a chronic exposure framework at levels approximating human exposure or at least at levels found within the environment. One needs to be aware that the lower the level of exposure, the more likely it is that individual differences in metabolism or other physiologic factors may bias the behavioral data.

A third variable is synergism, the effects between toxicants to which the test subjects are either experimentally or environmentally exposed. Currently, this problem can be conservatively and practically handled on a small scale with a factorial experiment using two or three toxicants. However, the synergism problem becomes more difficult or bewildering when considering the possible interactions between toxicants, inter-intra species specific effects, and the dose-response curve.

Although many physiologic experiments may not attempt to account for these variables, the very nature of behavioral toxicologic research necessitates their not being taken lightly. Each of these factors may be highly correlated not only with one another, but also with the total functioning of the organism both in and out of the experimental environment.

A fourth variable of considerable interest concerns the critical period of exposure. Regardless of when an organism is exposed to a toxicant, the effects observed are the result of many interacting variables, some of which are determined by the state of development of the organism. As earlier emphasized, alterations following a toxicosis at one point in the life span of the organism may be specific to that period only or may appear in other developmental stages. It is important, therefore, to examine carefully experimental data in light of developmental variables prior to (1) extrapolating findings from one period of development to another or (2) assessing the hazard of a toxicant.

Finally, there are insufficient data even to suggest what effects environmental factors such as bedding, frequency of cage cleaning, temperature and climatic variations, amount of handling, conditioning prior to experimentation, and housing might have on the outcome of an experiment. Obviously these are factors which, for the most part, we take for granted and place with our experimental error. However, when these factors are combined with those discussed above, they have serious implications for the data we produce from our experimentation and the methods we utilize in obtaining the data. Recent reviews (Fouts, 1976; Vesell *et al.*, 1976; Lang and Vesell, 1976) document the significance of these variables. A further discussion of methodological problems is provided by Bornschein and Michaelson in Chapter 4.

Predictions

Having attempted to describe some of the disciplines involved in the field of behavioral toxicology, some of the methods employed by these disciplines, and the variables which are potentially critical in the utilization of these methods, it seems appropriate to ascertain what directions research may take in the future. As our field of investigation matures, we may expect some fairly dramatic shifts in areas of study, changes in methodology, and a better delineation of the concepts involved in behavioral toxicology. Therefore, we feel confident that the future will see:

1. Continuation and refinement in the standardization of testing procedures with a goal of establishing a battery of sensitive behavioral tests for use by regulatory agencies in evaluating hazards of potential neurotoxicants. The emphasis here will probably be to develop the battery so that the tests provide information on a number of psychological capabilities, any one of which may be affected by the toxicant.

2. Increased use and improvement of automated and nonautomated behavioral testing techniques. Toxicologic research will more frequently use behavior as an index as techniques are improved and then will attempt to examine behavioral functioning from both individual and social perspectives.

3. Increased studies on the synergistic effects of neurotoxicants, not only with respect to other neurotoxicants, but also in relation to species effects and specific environmental or laboratory influences on behavior.

4. More studies of the critical times of exposure during the developmental stages of the CNS. Of particular interest will be studies linking specific psychologic deficits with specific periods of exposure.

5. Increased emphasis upon correlating behavioral changes with concomitant physiopathologic changes. Researchers in our field will someday be able to detect physiopathologic changes and reliably predict their behavioral correlates, and vice versa. The capability lies in the future, but the seeds are currently being planted.

6. Greater emphasis on the importance of theory in determining the direction of research. We are only beginning to abandon the "knee-jerk" approach to our research whereby we conduct experiments without regard for what has gone before both behaviorally and physiologically. With the advent of sound theory development generating testable hypotheses, behavioral toxicologic research will assume direction and, therefore, long-range goals.

7. More emphasis on the knowledge of the target area of toxic effect.

8. More attention to interpretive fidelity, to establishing stronger criteria for reliance upon behavioral toxicologic data before regulatory action is undertaken. The end result will be an elimination of the "straw-man" which may incorrectly support or deter the use of a toxicant.

Although these predictions are not all-inclusive, we feel they do represent developing interests among the growing group of people engaged in behavioral toxicologic research. Progress in behavioral toxicology will undoubtedly come from the strength of a diversity of disciplines and their contributions to methodology and theory. It is a well intergrated interdisciplinary approach which will ultimately provide the answers to the problems facing behavioral toxicology.

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3. A REVIEW OF THE DEVELOPMENTAL MODELS EMPLOYED IN BEHAVIORAL TOXICOLOGY

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Introduction

In assessing the effects of a potential toxicant on the developing offspring, one of the first questions to confront the investigator is "When should exposure occur?" Few colleagues have addressed this question to any extent (Spyker, 1975); and although administration of toxicants has occurred at almost every developmental period, it has rarely been done in any factorial manner within a study. Furthermore, although authors have discussed the methodological considerations in this general area of developmental psychopharmacology (Young, 1967; Brimblecombe, 1968; Kornetsky, 1970), they have essentially avoided this issue.

In many studies the primary concern of the experimenter is to demonstrate or reveal an "affected" organism, and it is often nor clear to the reader why a particular period of exposure was selected for study. Although this omission does not reduce the significance of the findings, an equally important issue should be the delineation of the periods in the lifespan of the organism during which toxicant exposure is necessary and/or sufficient to produce deleterious effects. Assessment without such information may be incomplete, and the availability of such information is of obvious value for prophylaxis.

The present chapter presents a review of the periods of development that have been examined for lead, mercury, and pesticides (the review covers only published articles and omits abstracts). Many of the considerations and directions

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suggested here also apply to that area of developmental psychopharmacology. Since this paper is primarily concerned with the consequences of the toxicant on the developing offspring, only a sampling of studies which administer the toxicant postweaning are presented. This procedure has been adopted primarily to provide the reader with some insight regarding the ages of the adolescent or adult organism at which each of the toxicants has been studied. In addition, the paper concentrates on behavioral studies outside the realm of electrophysiology/neurophysiology.

Developmental Models of Mercury Exposure

Prior to detailing studies on this toxicant, a brief explanation of the tabular format is necessary. In Table 3-1, which will serve here as an example, the fourth column, *"Parent,"* indicates which parent was exposed to the toxicant; the *"Premate Exposure"* column reflects whether or not the parent was pre-exposed to the toxicant prior to mating and the duration of such exposure; the *"Gestation Exposure"* and *"Nursing Exposure"* headings reflect exposure during those periods with the time of exposure indicated. Horizontal lines continuing through columns reflect continued exposure over more than one developmental period; in the *"Postweaning Exposure"* column, the day indicated reflects when treatment, not behavioral testing, was initiated. In this column, studies were grouped under *"Adult"* if only the weight range of subjects was provided with no indication of age.

Most of the behavioral studies on mercury have employed postweaning exposure, including a large number of studies on monkeys (e.g., Evans *et al.*, 1974; Helberg and Nystrom, 1972; Vitulli, 1974; Berlin *et al.*, 1973; and Berlin *et al.*, 1975) and birds (e.g., Beliles *et al.*, 1967, 1968; Rosenthal and Sparber, 1972) not included in this review. Most of the gestation-only studies exposed the pregnant female during critical days of organogensis. Thus Hughes and Annau (1976) orally dosed pregnant females on day 8 of gestation, Sobotka *et al.* (1974) intubated mothers on days 6-15 of gestation, while Spyker *et al.* (1972) administered intraperitoneal injections to pregnant mice on days 7 or 9. Both the Sobotka *et al.* (1974) and Spyker *et al.* (1972) studies point toward the sensitivity of the neuromotor systems to mercury. Sobotka *et al.* (1974) revealed that both eye opening and clinging ability developed earlier in neonates of mercury-exposed dams, while Spyker *et al.* (1972) found both differences in open-field behavior and disrupted swimming ability of mercury offspring.

In terms of assessing contribution of *in utero* exposure, both of these studies were confounded in terms of mercury carryover into nursing. Cross-fostering procedures have been employed to partition out this contribution (Zenick, 1974; Spyker, 1975; Hughes and Annau, 1976). Spyker found that survival rate was enhanced if offspring exposed during gestation were nursed by control mothers (74.4 percent) as compared to noncross-fostered counterparts (57.3 percent). This result suggests that the residual mercury carried over in the mother's milk was sufficient to have adverse consequences for that offspring. This deleterious, residual effect was further confirmed by discerning a depressed weaning weight in young born of control mothers and cross-fostered to treated mothers. In addition, motor disturbances became increasingly evident with age in both prenatal and postnatal exposed groups. No

Table 3-1. DEVELOPMENTAL MODELS OF MERCURY EXPOSURE

Author	Species	Route	Parent ♂ ♀	Premate exposure	Gestation exposure	Nursing exposure	Postweaning exposure (days of age)								
							30	40	50	60	80	100	120	150	Adult
Salvaterra *et al.*, 1973	M[a]	ip	–	–	–	–		42							
Morganti *et al.*, 1974			–	–	–	–		42							
Morganti *et al.*, 1976			–	–	–	–		42							
Klein & Atkinson, 1973	R[b]	Diet	–	–	–	–	25								
Klein *et al.*, 1974	R	Water	–	–	–	–	25								X
Braun & Synder, 1973		ip or iv	–	–	–	–									X
Sobotka *et al.*, 1974	R	Oral	♀	–	6——— 25										
Hughes & Annau, 1976	M	Oral	♀	–	8										
Spyker *et al.*, 1972	M	ip	♀	–	7;9										
Spyker, 1975					7;9;12;13										
Post *et al.*, 1973	R	Oral	–	–	–	15;21				60					
Zenick, 1974; 1976	R	Water	♀	–	1——— 21										
						1———— 21									
							22-30								
Olson & Boush, 1975	R	Diet	♀	–	1———————	———————	———	———	———	68					

[a] Mice

[b] Rats

behavioral data other than neuromuscular assessment was reported on these older animals. On the other hand, Zenick (1974) and Hughes and Annau (1976) found no effects on offspring weight or behavioral tasks in the cross-fostered young exposed only to this residual; however, these studies did confirm the detrimental influence of *in utero* exposure. In the Hughes and Annau study (1976), mice exposed to methylmercury *in utero* exhibited deficits in a two-way active avoidance shuttle box and in a punishment situation. No differences were observed on a conditioned suppression task, in an open field, or in a water-escape runway. The failure to see effects on the latter two tasks contradicts observations made by Spyker *et al.* (1972). Hughes and Annau (1976) also tested the mothers of the progeny as well as offspring of the progeny themselves. Neither group of subjects exhibited deficits, suggesting that mercury had more obvious deleterious consequences during *in utero* exposure leaving the treated mother unaffected; and, furthermore, the effect was restricted to the generation in which exposure occurred.

Zenick (1974) contrasted the effects of mercury exposure administered to independent groups at different periods of development. Potential mothers were exposed either during gestation or while nursing (treatment begun at parturition), or offspring were directly exposed beginning at weaning, day 21. As noted above, cross-fostered groups were also employed. Results indicated that offspring of mothers exposed during gestation and offspring exposed directly to mercury at weaning exhibited learning deficits on a brightness discrimination task begun at 30 days of age. These deficits also appeared in a retest session 21 days later. Offspring of mothers exposed during nursing only and offspring exposed only to the residual mercury in the milk were not different from controls on the learning task. However, in a subsequent study in which visual evoked potentials were recorded (Zenick, 1976), the nursing-only offspring were relegated to the "affected" category. The evoked potential data reinforces the concept of employing a battery of tasks in assessing critical periods of exposure and suggests that the sensory evoked potential may be as sensitive, if not more so, than other behavioral assessment techniques.

The effect of exposure during nursing, as contrasted with postweaning, has also been assessed by Post *et al.* (1973) who forcefed a single dose of mercury to either 15-, 21-, or 60-day old rats. No changes in discrimination learning or open-field behavior were observed in the nursing-exposed pups, while postweaning-exposed rats (60 days of age) were significantly different from controls on both tasks. These findings are in agreement with Zenick (1974).

Olson and Boush (1975) examined the effects of prolonged-multiperiod exposure within the same animal by feeding mercury to female rats throughout gestation and nursing, then maintaining the pups on the same diet throughout behavioral testing at 68 days of age. These mercury offspring exhibited retarded maturation of swimming behavior and the righting reflex and made significantly more errors than controls on a symmetrical maze task.

Data on mercury seem to suggest that exposure during gestation or postweaning is sufficient to produce an effect on a number of tasks. The influence of early postnatal exposure via the mother's milk is still uncertain; however, in conjunction with gestation exposure, treatment during nursing may create the most potentially hazardous setting for the offspring.

Developmental Models of Lead Exposure

A greater variety of developmental models have been employed in examining the behavioral effects of lead exposure than in studying either mercury or pesticides. There are at least two reasons for this situation: the adult animal has not served as a good model for examining the effects of low-level lead exposure because the adult exhibits extreme resistance to the CNS effects of lead poisoning, requiring very large dosage of lead to produce even slight clinical manifestations of CNS toxicity. Secondly, because of its early widespread use, lead toxicity via pica posed a very real environmental threat for the developing child, especially those living in older, substandard housing.

Frequently employed developmental models are shown in Table 3-2. Although no lead study has recorded behavioral measures on pups exposed *in utero* only, a suggested differential sensitivity of this period as compared to nursing may be gleaned from a study by Snowden (1973). In that experiment, mothers were injected ip with lead either thoughout pregnancy or throughout nursing, or pups were directly exposed to lead by injections for 37 days beginning at 22 or 100 days of age. The postweaning injections had no effects on maze learning. However, females injected during pregnancy did not give birth to viable offspring; whereas the same dosage administered to mothers during nursing did not produce any fatalities, but it did lower the pups' body weights and increase their errors on a maze task. Although there may be a confounding between route of exposure, namely ip injection, and critical period, the study does suggest a need to examine more closely the contribution of gestation exposure in terms of increased sensitivity to lead.

A developmental model developed by Pentschew and Garro (1966) has become the prototype for studying developmental lead toxicity. These authors were able to produce CNS lesions in pups sucking a dam exposed to 4-5 percent lead carbonate in her daily diet. Unfortunately, direct access to the maternal diet by the pups was possible at approximately 18 days of age, resulting in the appearance of clinical symptoms (e.g., retarded body weight, paralysis, etc.) in the offspring at 20-30 days of age. A procedure to correct this methodological flow and incorporate other needed controls has been presented by Michaelson and Sauerhoff (1974a, b). However, the Pentschew and Garro model (1966) has left a definite impression as witnessed by the large number of papers employing the nursing exposure model to assess the effects of lead (Table 3-2). Many investigators, therefore, assume that the oral ingestion of lead (via milk) is analogous to the pica route of lead-containing materials by human infants (Michaelson and Sauerhoff, 1974a).

Sobotka and his colleagues (1974, 1975) have taken a somewhat different approach to nursing exposure in that they directly intubated the pup beginning at day 2-3 and continued this exposure until weaning. Direct intubation of pups during nursing has also been done by Golter and Michaelson (1975). Pups exposed to lead exhibited an attenuated response to amphetamine-induced

Table 3-2. DEVELOPMENTAL MODELS OF LEAD EXPOSURE

Author	Species	Route	Parent ♂ ♀	Premate exposure	Gestation exposure	Nursing exposure	Postweaning exposure (days of age) 30	40	60	80	00	120	150	180	Adult
Avery *et al.*, 1974	R[a]	Oral	—	—	—	—									X
Shapiro *et al.*, 1973	R	Ip	—	—	—	—					90				
Bullock *et al.*, 1966	R	Ip	—	—	—	—									X
Terzin & Vujkov, 1968	M[b]	Ip	—	—	—	—						120			
Sobotka & Cook, 1974 Sobotka *et al.*, 1975	R	Oral	—	—	—	3––––21									
Brown, 1975	R	Water, oral, or Ip (pup)	— ♀	—	—	1––––––21 1–––10 11––21									
Snowden, 1973	R	Ip	—	—	1––––21	1––––21	22					100			
Brown *et al.*, 1971	R	Ip	—	—	—	8 21		35							

(continued)

Table 3-2. DEVELOPMENTAL MODELS OF LEAD EXPOSURE (continued)

Author	Species	Route	Parent ♂	Parent ♀	Premate exposure	Gestation exposure	Nursing exposure	Postweaning exposure (days of age) 30	40	60	80	00	120	150	180	Adult
Sauerhoff & Michaelson, 1973																
Michaelson & Sauerhoff, 1974a,b	R	Diet	–	♀	–	–	1----------25									
Golter & Michaelson, 1975	R	Diet or oral (pup)	–	♀	–	–	1------------35									
Silbergeld & Goldberg, 1973, 1974a,b, 1975	M	Water	–	♀	–		1-----21 1---------------60									
Driscoll & Stegner, 1976	R	Water	–	♀	–	1----------------31-----70-----------180										
Zenick *et al.*, 1978a,b	R	Water	–	♀	60 days	1------------21										
Padich & Zenick, 1977	R	Water	–	♀	30 or 60 days	1--------------------42 1--------------21	22----42									
Reiter, *et al.*, 1975	R	Water	♂	♀	40 days	1--------------------------------120										
Brady *et al.*, 1975	R	Oral	♂ – ♂ –	♀ ♀ –	60 days	1--------------21 1--------------21										

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[a] Rats

[b] Mice

increases in activity and deficits in a two-way shuttle box (Sobotka and Cook, 1974) and in a habit-reversal task (Sobotka *et al.*, 1975). Sobotka has suggested that the advantages of this technique were that "this approach avoids the confounding variables of maternal effects of lead and also affords a more effective means of determining the dose of lead that each pup receives (Sobotka *et al.*, 1974). However, the technique does have its disadvantages in that it results in the delivery of the lead dosage as a single bolus into the stomach, altering the parameters of absorption from that which would be observed with a slower rate of intake spread over a greater period of time each day. Furthermore, we have observed that intubation can be stressful to the animal (Brady *et al.* 1975), and this stress may have an indirect, differential effect on lead-treated pups.

Brown administered lead during nursing either to the mother, by gavage or in her drinking water, or to the pup directly, ip. Irrespective of route, Brown (1975) found a significant performance deficit on a T-maze discrimination task when pups were tested at 8-10 weeks. Furthermore, by contrasting groups exposed during days 1-10 with pups exposed during days 11-12, Brown noted that exposure was necessary during the first 10 days in order for lead to exert a detrimental influence. Since blood lead levels were significantly higher in the 1-10 day old pups, as compared to 11-20 day lead pups or controls, Brown (1975) suggested that the increased sensitivity of this group may, in part, be a result of age-related differences in uptake, distribution, and retention of lead.

The influence of nursing exposure has also been investigated by Michaelson and coworkers (Sauerhoff and Michaelson, 1973; Michaelson and Sauerhoff, 1974a; Golter and Michaelson, 1975; Bornschein *et al.*, 1977). These studies, however, were not nursing-only exposure in the truest sense because the pups were continued on the treatment following weaning and throughout testing. It is possible that this direct exposure at what was still a relatively young age (21 days) may also have exerted detrimental effects. Aside from this consideration, Michaelson's group found changes in activity levels of the lead-exposed offspring as compared to controls. However, they also noted that an interaction of lead and a state of undernutrition in the pup (induced by the decreased food intake by the lead mother) must be considered in interpreting the data (Bornschein *et al.*, 1977). In fact, this retarded body weight of pups appears consistently across laboratories that employ dosages in the range of the Pentschew and Garro study (1966).

The opportunity to contrast the combined nursing and early postweaning exposure with nursing-only exposure has been investigated by Silbergeld and colleagues in a series of papers. Her treatment groups consisted of pups exposed to lead only while nursing via the mother's milk or pups which were exposed while nursing as well as after weaning, throughout testing. While Silbergeld reported a decreased weight in the former group until it was switched to the control fluid, the report on the behavior of these offspring is still not clear: "Motor activity was also measured in offspring removed from the lead solutions of 10 mg/ml at weaning. Four out of the six mice measured showed increases in motor activity comparable to those seen in mice maintained on lead. However, two of these six mice had activity levels not significantly higher than those of controls" (Silbergeld and Goldberg, 1973). A similar statement on this group's behavior was made in a second study (Silbergeld and Goldberg, 1974) which rather than being a replication of earlier work, involved an additional manipulation made on the same animals. It is apparent that additional work with the group exposed only during nursing (and larger N) is necessary to confirm the deleterious effect of such exposure.

The question of developmental versus direct postweaning exposure has also been addressed in a study by Padich and Zenick (1977) which employed four groups: Group Pb/Pb, mothers pretreated with lead and continued on lead during gestation and nursing with the offspring continued on lead after weaning and throughout testing (developmental and direct exposure); Group Pb/C, offspring developmentally exposed only; Group C/Pb, offspring exposed directly only; and Group C/C, no lead exposure. Significant differences were found between Groups Pb/Pb and C/C on a fixed ratio (FR20) bar press task; however, exposure of the offspring during gestation and nursing only (Group Pb/C) or during postweaning only (Group C/Pb) did not result in any impaired performance on the FR task, nor was their performance different from controls under fixed-interval schedules (Zenick *et al.*, 1978a). However, the Pb/C offspring have been shown to be inferior to controls on discrimination learning tasks (Brady *et al.*, 1975; Zenick *et al.*, 1978b). Thus it appears that for some tasks a combined developmental/direct exposure is necessary for lead to exert an effect. As suggested earlier, this may contribute, in part, to the effect seen by Michaelson in animals continued on treatment following nursing-exposure (Michaelson and Sauerhoff, 1974a; Sauerhoff and Michaelson, 1973; Golter and Michaelson, 1975). In any event, these findings suggest that any conclusions regarding critical exposure periods and lead susceptibility must be qualified with regard to the instrument of measurement.

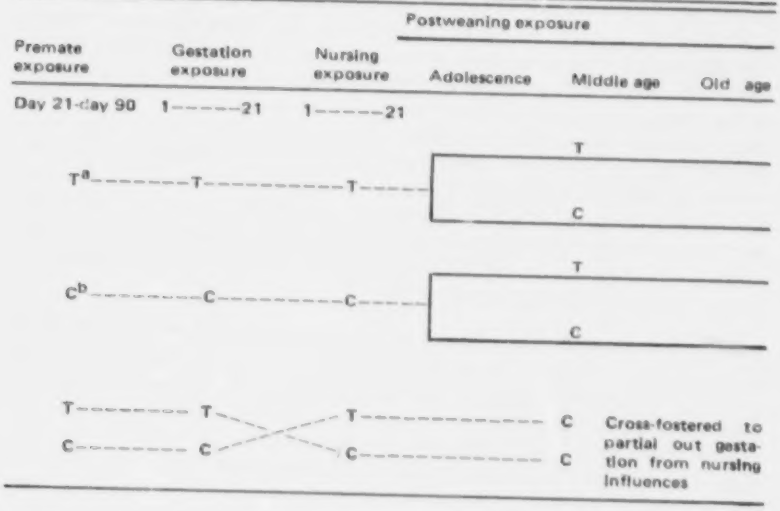
Encompassed in a large number of developmental lead studies have been some unique experimental manipulations rarely reflected in the study of other toxicants. These manipulations include assessing the effects of prolonged maternal exposure (Driscoll and Stegner, 1976; Reiter *et al.*, 1975a; Brady *et al.*, 1975; Padich and Zenick, 1977; Zenick *et al.*, 1978a, b) and the contribution of paternal exposure (Brady *et al.*, 1975). Driscoll and Stegner (1967) have assessed the influence of chronic exposure by administering lead throughout gestation and nursing while Reiter *et al.* (1975) and Zenick *et al.* (1978a, b) have extended this period backward to include parental exposure prior to mating. In Reiter's laboratory, this "premating exposure" incorporated pretreatment for a period of 40 days to allow lead concentration to approach steady states. This objective has been included in the rationale employed in our laboratory; however, it is only a part of a philosophy which assumes that the potential mother may have existed in the toxic setting for some time prior to mating, conceivably her entire life. As a consequence, there may be a buildup and storage of the toxicant in her system, resulting in another potential source to which the fetus or pup may be exposed in addition to the mother's daily dosage. Subsequent to mating, the mother continued in this environment during gestation and nursing. This hypothesis has led to the current model employed in our laboratory that is presented in Table 3-3. Such a model incorporates premating exposure while contrasting developmental versus direct toxicant exposure on offspring behavior. A cross-fostering manipulation is also included. In addition, assessment is made throughout the life of the animal in order to evaluate permanence as well as detect any late-occurring effects.

Another manipulation in studying developmental lead toxicity has been the attempt to partial out the parental contribution. A study conducted by Brady *et al.*, (1975), employing a pretreatment model, contrasted the effects of exposing both potential parents to lead with exposure of the mother or father only. Analysis of offspring behavior on a water-escape T-maze task revealed that the three lead groups made more errors than the controls but did not differ from one another. However, offspring resulting from dual parental exposure had longer swimming times than the single-parental exposure groups who, in turn, were slower than the controls. The finding of a paternal-only effect has since been replicated in our laboratory. The ramifications of such a finding may be far-reaching. For instance, limitations on employment of pregnant women in industry are common; however, the Brady *et al.*, (1975) findings suggest that the standards for men may also need further consideration. At the very least, the question of paternal influence merits further investigation.

Although not reported in Table 3-2, there have been a number of behavioral studies examining lead toxicity which have employed species other than rodents as subjects, the most notable effort being a series of investigations by Van Gelder and colleagues employing sheep as an experimental model. They examined the effects of lead administration to the adult (Van Gelder *et al.*, 1973), exposure *in utero* plus carryover (Carson *et al.*, 1974a), and early postnatal treatment (Carson *et al.*, 1974b). Employing a series of measures, the group has found deficits primarily on a visual discrimination task in lambs that had been exposed *in utero*. These animals were tested at 10-15 months of age subsequent to prenatal exposure, reflecting a disruption of behavior in the absence of elevated blood lead levels, suggesting that lead exposure during gestation is sufficient to induce permanent debilitation. This aspect of the persistence of an effect in the absence of elevated toxicant levels is a critical issue that has been neglected by researchers in this area. This point will be addressed more directly in the final section of this chapter.

Finally, a study with infant monkeys by Allen *et al.* (1974) should be mentioned, since one phase of the study closely simulated lead poisoning via pica. In that study, infant rhesus monkeys were housed for three months with surrogate mothers having a lead base (other infants received lead via their formula). As Allen *et al.* (1974) notes, "throughout this period the infants scratched, licked, and rubbed the lead bases," an interaction with their environment that closely mimics pica. The research team observed neuromuscular and visual impairments and altered social behavior with the latter symptom persisting when external lead exposure was eliminated. These effects were not observed in adolescent or adult monkeys receiving similar dosages. These results again reinforce that view of the increased susceptibility of the developing organism to low-level exposure as contrasted with its adult counterpart.

Table 3-3. A MODEL TO CONTRAST DEVELOPMENTAL/DIRECT EXPOSURE


Postweaning exposure
Premate exposure
Gestation exposure
Nursing exposure
Adolescence
Middle age
Old age
Day 21-day 90
1-----21
1-----21
T^a
T
T
T
C
C^b
C
C
T
C
T
T
T
C
C
C
C
C
Cross-fostered to partial out gestation from nursing influences

^{a}T = Toxicant.

^{b}C = Control.

In summary, although there have been a number of developmental studies of lead toxicity, many questions remain unanswered, especially the contribution of *in utero* exposure. Future use of cross-fostering techniques may shed some light on the influence of gestation exposure alone. Furthermore, there is a need to do more extensive long-term chronological assessment.

Developmental Models of Pesticide Exposure

Review of Table 3-4 will reveal that the number of developmental studies on the effects of pesticides on behavior is scant compared to the number of investigations of the heavy metals. This dearth of developmental work is rather paradoxical, since initial concern over pesticide toxicity has given the overall area of environmental toxicology possibly its greatest impetus. Many of the studies of pesticides examined only their anticholinesterase properties for delineating the role of acetylcholine in the CNS and have ignored their toxic effects. There are several articles available on these studies, the most recent being a review by Bignami *et al.* (1975). Since these studies have been done almost exclusively in adult animals, they will not be considered in this chapter.

The influence of DDT and Parathion exposure during gestation has been systematically studied by Al-Hachim and Fink (1967; 1968a, b, c). In their investigations, groups of female rats were exposed to DDT during different trimesters of pregnancy. They observed a delay in the acquisition of a conditioned avoidance response in offspring of mothers treated during either the second or third trimester of pregnancy (Al-Hachim and Fink, 1968b). However, since the animals were tested at 30 days of age, Al-Hachim suggested that this differential effect may have been a result of greater opportunity for the accumulation of DDT (via placenta and/or mother's milk) in offspring exposed at later points in gestation, since they failed to see effects of DDT on open-field behavior (Al-Hachim and Fink, 1968c) in animals tested after post-weaning (60-66 days and 70-90 respectively). Unfortunately, rather than employing different tasks, it would have been necessary to assess conditioned avoidance responding in older animals before contributing only a transitory, biochemical effect to gestational DDT exposure. It is possible that the later stages of pregnancy do reflect an increased vulnerability above and beyond the simple fact of increased accumulation of DDT.

In the only factorial manipulation of developmental pesticide exposure, Craig and Ogilvie (1974) contrasted the effects of exposure during gestation and nursing with exposure to DDT during either gestation or nursing only. This later manipulation was achieved by employing cross-fostering procedures. One of the points which was not clear from the study was whether the DDT mothers remained on treatment during nursing or whether treatment was discontinued at birth so that nursing-only exposure more accurately reflected a gestation carryover; (the cross-fostering design is addressed in greater detail in the next section. Interestingly, Craig and Ogilvie (1974) found that DDT administered during either gestation or nursing resulted in offspring performance that was either equivalent or superior to controls on a series of dependent measures of maze learning. On the other hand, the combined gestation-nursing exposure resulted in inferior performance as compared to controls.

One conclusion that might be drawn is that DDT is toxic only if exposure occurs during both gestation and nursing. However, Craig and Ogilvie (1974) forwarded the hypothesis that the differing performances were a result of a dose-related, biphasic effect of DDT. Essentially, since the single periods were of shorter duration, the offspring were exposed to a cumulatively lower dosage than their dual-period-exposure counterparts. Craig and Ogilvie (1974) suggested a biphasic phenomenon, with lower dosages of DDT (gestation or nursing only) being stimulatory and higher dosages (gestation and nursing exposure) interfering with performance. This dose-response hypothesis is similar to that noted above by Al-Hachim. However, one point that should be noted is that Craig and Ogilvie (1974) began testing their rats at 49 days of age and retested them one month later. Since treatment was discontinued at weaning, it was possible that the concentration of DDT was extremely low or absent during testing. The ability of the compound to exert a stimulatory or inhibitory influence in light of this is puzzling. However, the data are suggestive of a need to more closely examine the relationship between dose and period of exposure. Furthermore, this study is one of the few where an attempt was made to assess permanence of an effect via a test-retest design. (See the following section).

Table 3-4. DEVELOPMENTAL MODELS OF PESTICIDE EXPOSURE

								Postweaning exposure (days of age)							
Author	Pesticide	Species	Route	Parent ♂ ♀	Premating	Gestation	Nursing	30	40	60	80	100	120	150	Adult
Pearson *et al.*, 1969															
Clark & Pearson, 1973	Disyston	R	Diet	–	–	–	–	30							
Clark *et al.*, 1971	Disulfoton	M	Diet	–	–	–	–								X
Russel *et al.*, 1961	Systox	R	Diet	–	–	–	–		42						
Reiter *et al.*, 1973	Parathion	M	Intubate	–	–	–	–				100				X
Khairy, 1959	DDT	R	Diet	–	–	–	–								
Peterie & Peterie, 1971	DDT	M	Diet	–	–	–	–					120			
Sobotka, 1971	DDT	M	Intubate	–	–	–	–					130			X
Al-Hachim & Fink, 1967	DDT Parathion	M	Intubate	♀	–	1-7 7-14									
Al-Hachim, 1971	Aldrin	M	Intubate	♀		14-21 14-21									
Craig & Ogilvie, 1974	DDT	M	Diet	♀	–	1------------ 1----21^a	-----21 1----- 21^a								
Woolley, 1970	DDT	R	Diet	♀	x------ (Several days?)	------------	------------	------	------	--84					

^{a}Cross-fostered.

There has been only one prolonged developmental exposure study using DDT (Woolley, 1970) in which potential mothers were exposed prior to mating with exposure continued throughout gestation and nursing. The offspring were then maintained on the treatment for the duration of the study. Unfortunately, only limited behavioral assessment was done, with the main effect of DDT being to counteract the ability of strychnine to increase the intensity of electroshock seizures.

In addition to the rodent, a number of other species have been employed in assessing the effects of pesticides in postweaning animals. Several studies have used the monkey (Reiter *et al.*, 1975b; Smith *et al.*, 1976; Lattal and Wilber, 1971; Revzin, 1970) and a series of studies in sheep have been summarized by Van Gelder (1975).

This review of pesticide literature reinforces the urgency to gain greater insight into the developmental influences of pesticides. The small number of developmental studies, limited dose-response data, and poverty of behavioral measures make conclusions regarding the developmental consequences of pesticide exposure premature.

Future Directions in Developmental-Behavioral Toxicology

Although there have been only a few studies which have employed cross-fostering techniques (Zenick, 1974, 1976; Spyker, 1975; Hughes and Annau, 1976; and Craig and Ogilvie, 1974), the necessity of such a manipulation is essential in evolving a more complete picture of the interaction of a particular toxicant with the various periods of development. The design for cross-fostering and foster-rearing has been succinctly described by Spyker (1975) and in essence consists of placing pups at birth with mothers of opposing treatments (cross-fostering) or same treatment (foster-rearing), the latter being a control manipulation. Other pups may remain with their natural mothers. Such manipulations may not only partial out the influence of gestation versus nursing exposure, but also separate the direct effects of the agent on the offspring from effects on maternal behavior towards the offspring. Such a manipulation provides a clearer concept of necessary and/or sufficient periods during which toxicant exposure can have adverse consequences for the offspring. This information is vital for prophylaxis. The nature of the prophylactic treatment will vary depending on whether exposure during gestation only, nursing only, or both periods is sufficient to cause damage to the offspring.

Although the cross-fostering manipulation appears relatively straightforward, there is some inconsistence in the manner of treating the treatment mother. Most studies cease treatment for all mothers at parturition. Subsequently, pups cross-fostered on a treatment mother are exposed only to residual toxicant carried over from gestation. This arrangement is different from ones involving treated mothers maintained on the toxicant during nursing. Studies employing the "residual" model cannot as readily conclude in the face of negative findings that nursing exposure does not exert an effect. The reduced dosage and length of exposure may mask an effect that would appear with actual, full nursing exposure. Future researchers addressing the question of critical periods need to be alerted to the differences that may be obtained depending upon which cross-fostering technique is employed.

In terms of assessment there appears to be another gap in our research efforts. Table 3-5 contains a list of studies that have employed either chronological or test-retest assessment and/or have run toxicant determinations for the purpose of testing the animal in the absence of elevated levels of the toxicant. The brevity of this list is quite discouraging although some studies have been omitted where testing was delayed long enough to assume the absence of the toxicant (e.g., Sobotka *et al.*, 1975; Al-Hachim, 1968a, c; and Hughes and Annau, 1976).

Table 3-5. STUDIES USING CHRONOLOGICAL OR TEST-RETEST AND/OR TESTING IN THE ABSENCE OF HIGH TOXICANT LEVELS

Toxicant	Chronological or test-retest	Behavioral deficits in absence of compound
Mercury	Spyker, 1975 Zenick, 1974, 1976	Zenick, 1974, 1976
Lead	Sobotka *et al.*, 1975	Brown, 1975 Carson *et al.*, 1974b
Pesticides	Craig and Ogilvie, 1974	------------

The value of chronological assessment is essential, for it is conceivable that certain disturbances may be delayed or lie dormant only to appear later in life. This situation has certainly been documented for mercury (Spyker, 1975). On the other hand, deficits revealed in young may disappear with age. Reiter *et al.* (1975) has suggested that a maturational lag may account for such behavior. The test-retest design also fits into this scheme in terms of reflecting permanence of a deficit.

In conjunction with these experimental manipulations, analyses of toxicant levels need to be conducted at various times during testing. As long as the compound persists in the system, the possibility of a reversible, biochemical lesion exists. Deficits persisting in the absence of the compound suggest a more pathological and likely irreversible effect.

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4. METHODOLOGICAL PROBLEMS ASSOCIATED WITH THE EXPOSURE OF NEONATAL RODENTS TO LEAD

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Introduction

The more dramatic, harmful effects of pediatric lead encephalopathy are well recognized. Ample supporting evidence of a dangerous body burden can be found whenever the blood lead exceeds 80 to 100 μg lead per 100 ml. The central nervous system is a prime target following acute high level exposures. Inhibition of heme synthesis and proximal tubular injury can also be detected.

Until recently lead poisoning has been defined as a public health problem only for those children living in inner city metropolitan slum areas. However, the definition of the population at risk needs re-evaluation. The concentration of inorganic lead in our immediate environment is on the increase (Patterson, 1965). Young children are being chronically exposed to low levels of lead in their diets, in the air they breathe, and in the soil and dust which are found in their play areas (Cohen *et al*., 1973; Vostal *et al*., 1974). This chronic low-level exposure is being reflected in elevated blood lead levels in the range of 30 to 60 μg/100 ml (Caprio *et al*., 1974). Furthermore, recent studies indicate that such elevated blood lead levels are not uncommon in children growing up in rural areas (Fine *et al*., 1972). Therefore the population at risk as a result of undue lead exposure is not restricted to just those children living in the inner city.

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A major question from the public health point of view is: What effect does excessive lead absorption have on mental development among those who never develop encephalopathy or any other overt symptoms of poisoning? At present there is good reason to believe that adverse effects may be particularly marked when exposure occurs during the period of central nervous system development in early childhood. For example, Byers and Lord (1943) reported retarded mental development in lead-poisoned children without encephalopathy, and Albert *et al.* (1974) found deficits in intellectual maturation in asymptomatic lead-exposed children. However, we have very little reliable information concerning the potential neurotoxic effects of chronic low-level lead exposures, even though there have been indications from clinical studies that such exposures during early childhood may produce behavioral and intellectual impairment (de la Burdé and Choate, 1972).

Clinical studies which attempt to derive data from asymptomatic pediatric populations are fraught with numerous methodological problems. For example, although a child may exhibit an elevated blood lead level at the time of examination, we usually have no indication of that past history of lead exposure, either with respect to the maximum level obtained, the duration of the lead exposure, or the timing of the exposure relative to various critical aspects of central nervous system development. Furthermore, we usually have no measure of the child's intellectual development prior to the onset of lead exposure. Because of these problems, there has been considerable research effort devoted to the development of an animal model for asymptomatic neonatal lead exposure. Most experimental animals are resistant to the effects of lead on the brain. A review of the literature on this subject shows an amazing resistance of the adult animals to lead poisoning (Pentschew, 1958). However, our work and that of others indicate that if lead is given to newborn rodents, they suffer impairment in central nervous system function.

Table 4-1 provides a brief overview of some of the animal models used to investigate the effects of lead on the developing nervous system. It is apparent that there is a great diversity in exposure parameters such as route, level, and duration as well as timing of exposure relative to age. Unfortunately, the blood, brain, and whole body burdens which result from these various exposure techniques have not been well documented. In addition, the range of behaviors examined in lead-exposed animals has been rather limited. This limitation is unfortunate since, as mentioned previously, animal models may provide insight into the relationships between level and timing of lead exposure and resultant body burden, behavioral effects, and duration of effects. The purpose of this paper is to identify some of the inherent methodological problems associated with experimental neonatal lead exposure. We also intend to suggest strategies which should be of use in overcoming some of these problems.

Methods of Lead Exposure

A common method of exposure is to treat the neonate by daily oral intubations, permitting a relatively accurate estimate of daily exposure. This technique has several inherent disadvantages, including: (a) delivery of a single

Table 4-1. PROTOCOLS USED TO STUDY EFFECTS OF LEAD ON ANIMAL BEHAVIOR

Reference	No.		Lactating dam	Neonate	Weanling	Adult	Pb concentration µg% Blood	Brain	Milk	Behavioral measures
Brown *et al.*, 1971	1	R			100[a]					Water maze and shuttle box learning
Brown, 1975	2	R		4.1[a]			198-257			T-maze learning, locomotor activity
Brady *et al.*, 1975	3	R	500[b]							Water maze learning
Golter & Michaelson, 1975	4	R		1[b]	40[c]		225	48		Locomotor activity
Michaelson & Sauerhoff, 1974	5	R	27,300[c]		25[c]			50	2500	Locomotor activity
Reiter *et al.*, 1975	6	R	5 & 50[d]		5 & 50[d]	5 & 50				Reflex development, locomotor activity
Shapiro *et al.*, 1973	7	R				0.16-0.96[a]				V.I. 30 sec operant discrimination
Snowden, 1973	8	R			5, 8, 12[a]	5, 8, 12[a]				Hebb-Williams maze
Sobotka *et al.*, 1975	9	R		5, 15, 44[b]			18-71			Locomotor activity, shuttle box learning
Morrison *et al.*, 1975	10	M	1090-5500[d]		1090-5500[d]	1090-5500[d]				Consumatory behavior
Silbergeld & Goldberg, 1973	11	M	1090-5500[d]		1090-5500[d]	1090-5500[d]				Locomotor activity
Terzin & Vujkov, 1968	12	M				7-36[a]				Locomotor activity
Carson *et al.*, 1974	13	S					5, 19, 35			Visual discrimination learning
Van Gelder *et al.*, 1973	14	S				55[b]				Auditory signal detection task
Allen *et al.*, 1974	15	P		2.1-5.1[b]			100-500			Social behavior
Goode & Calandra, 1973	16	P			0.05, 0.5 &5.0[b]	0.05, 0.5 & 5.0[b]	45-82			Delayed and conditioned response

[a] mg/kg of Pb ip
[b] mg/kg of Pb po
[c] ppm of Pb in diet
[d] ppm of Pb in water

R = Rat
M = Mouse
S = Sheep
P = Monkey

bolus of the agent with a short transit time through the gastrointestinal tract, (b) large periodicity in resultant blood lead concentrations, (c) trauma associated with daily forced feeding, and (d) considerable expenditure of time and effort by the experimenter. Our interest in chronic exposure and the detection of any resultant behavioral alterations precludes such a method of exposure. For these reasons we have chosen to expose neonatal rodents to toxic agents via the dam's milk. This approach is currently utilized by several investigators exposing rats and mice to inorganic lead (Brown, 1975; Maker *et al.*, 1975; Silbergeld and Goldberg, 1973; Golter and Michaelson, 1975), DDT (Fahim *et al.*, 1970), and methylmercury (Brown *et al.*, 1972). Although this indirect method of exposure mimics the usual oral route of entry into the body and avoids the disadvantages of oral intubation, it is difficult to estimate the daily lead exposure of the rapidly developing neonate. This difficulty in turn has hampered development of animal models with realistic levels of exposure relative to the human pediatric population. Furthermore, it has been difficult to compare this model to others using direct injection or intubation procedures.

Investigators who use the lactating dam as a vehicle for delivery of lead usually report only the concentration of the agent in the dam's drinking water or food (Brown, 1975; Maker *et al.*, 1975; Silbergeld and Goldberg, 1973; Golter and Michaelson, 1975). Few report milk lead concentrations (Michaelson and Sauerhoff, 1974; Golter and Michaelson, 1975). None have attempted to estimate the daily amount of lead ingestion by the suckling. While it is relatively easy to determine milk lead concentrations, it is much more difficult to estimate the amount of milk consumed by the suckling. There are several techniques available for estimating milk production by the lactating dam. These include: (a) determination of differences in weight between freshly dissected, milk-filled glands of dams separated from their litter for a fixed interval (usually 8 hours) and the mean weight of mammary glands from unseparated control animals at the same stage of lactation (Hanwell and Linzell, 1972), (b) biochemical determination of lactose content of mammary tissues in suckled versus nonsuckled lactating dams (Mutch and Hurley, 1974), and (c) maximum milk yield during artificial milking after a fixed interval of separation from pups (Hanrahan and Eisen, 1970). These techniques provide only an indirect estimate of how much the neonates may be ingesting. The most commonly employed technique for directly estimating milk consumption is to allow the pups to remove milk from the dam and then determine the amount suckled during a specified interval either by estimating the changes in body weight before and after suckling, or by removing and weighing the stomach and its contents at completion of the suckling period (Grosvenor and Turner, 1956, 1957, 1959; Grosvenor and Mena, 1967; Moon and Turner, 1959; Mena *et al.*, 1974). Another approach is to hand-feed (Miller and Dymsza, 1963) or infuse (Messer *et al.*, 1969) the neonates with normal rat milk and determine the quantity per 24 hours necessary for maintenance of a normal growth pattern. Intubation or infusion techniques probably result in an overestimate of actual milk consumption by normal suckling neonates. Overestimates can result from: (a) increased caloric requirements arising from excessive handling during artificial feeding, (b) ineffective utilization of caloric intake due to differences between artificial patterns of feeding and natural daily rhythms in suckling behavior (Grota and Ader, 1974; Redman and Sweeney, 1976), and (c) differences between the caloric values of infused or intubated milk, usually obtained during peak lactation periods (10 to 12 days, 1.3 Kcal/ml) by artificial milking and that milk obtained by neonates during natural suckling (approximately 1.9, 1.3, 1.5 Kcal/ml during weeks 1, 2, and 3 respectively) (Bornschein *et al.*, 1977a). It is important to emphasize that thermoregulatory mechanisms are not well developed in newborn rats and do not become operational until after the first week of life (Fowler and Kellogg, 1975). Close contact between litter and dam is necessary for maintenance of normal body temperature. The procedure of intubation or infusion upsets the normal litter-dam relationship, and this insult can be amplified by artificial feeding of late lactation (low calorie) rat milk, when milk normally produced in the early stages of lactation is of relatively high caloric content.

Because of these various methodological drawbacks, we sought a different approach to the estimation of daily milk consumption. As a result of this search, we have developed a way by which one can make a fairly good approximation of the volume of milk and, consequently, the amount of lead consumed each day (Fox *et al.*, 1975; Michaelson *et al.*, 1976; Bornschein *et al.*, 1977b).

The volume of milk consumed each day can be estimated with knowledge of: (a) the caloric requirements necessary to maintain normal daily growth during the suckling period and (b) the caloric value of milk during lactation. Estimated caloric requirements per gram body weight in neonatal rats range from 0.29 Kcal on day 1 to 0.53 Kcal on day 18. Caloric values of milk range from 2.62 Kcal/ml to 1.48 Kcal/ml on the 1st through 18th day of lactation. Neonates are highly efficient in extracting energy from the milk, the efficiency of utilization being over 90 percent. Estimated milk consumption per suckling pup increases from 0.7 ml on day 1 to 17.1 ml on day 18. Details pertaining to the above discussion have been published (Bornschein *et al.* 1977b).

A sample calculation appears below:

$$\text{Grams of milk} = \frac{\text{Body wt. (g)} \times \text{caloric requirement (Kcal/g)}}{\text{Caloric value of milk (Kcal/g)} \times \text{eff. utilization}}$$

For example, on day 5: Neonate's body wt = 12 g
Caloric requirement = 0.29 Kcal/g
Caloric value of milk = 1.25 Kcal/ml
Efficiency of utilization = 0.9

$$\text{Grams of milk} = \frac{(12\text{ g})\ (0.29\text{ Kcal/g})}{1.30\text{ Kcal/ml} \times 0.9} = 3.0$$

Our theoretical estimates of daily milk consumption are in good agreement with our expectation based on limited reports of milk production in rodents. We estimate that on day 10 of lactation a 300 gram rat nursing six pups yields 45 ml of milk in 24 hours. A recent study by Hanwell and Linzell (1972) on the rate of milk secretion in rats on the tenth day of lactation indicated that 240-280 gram rats produce 42 ml of milk per day. Thus, our estimates during late lactation are in general agreement with the limited data available. We recognize the need for experimental validation of our theoretical estimates and have conducted a 24-hour fostering experiment in which normal nonexposed sucklings received lead from an exposed lactating rat. Analytical estimation of the lead-load in these sucklings yielded 90 percent to 126 percent recovery relative to the expected value (Table 4-2).

Table 4-2. ACCUMULATION OF LEAD IN 12-13-DAY-OLD RATS FOLLOWING 24 HOURS OF EXPOSURE VIA CONTAMINATED MILK

Pup No.	A Body weight (g)	B[a] Milk consumed (g)	C[b] μg Pb consumed B x D	D[c,d] μg Pb/ml milk	E[c] Pb body burden μg (corrected)	F % Recovery E/C
1	28.7	10.3	14.9	1.45 ± 0.05[e]	15.8	106
2	30.6	11.2	16.2	1.45 ± 0.05[e]	15.1	93
3	28.7	10.3	14.9	1.45 ± 0.05[e]	14.0	94
4	27.1	9.7	23.8	2.45 ± 0.45[f]	24.5	103
5	30.9	11.0	27.0	2.45 ± 0.45[f]	27.8	103
6	29.8	10.9	26.7	2.45 ± 0.45[f]	26.6	100
						$\bar{X} = 100 \pm 5$

[a] Theoretical estimate.

[b] Predicted intake.

[c] Experimentally determined.

[d] Two milk samples per dam, each sample analyzed in triplicate.

[e] Milk lead content of lead-exposed dam No. 1.

[f] Milk lead content of lead-exposed dam No. 2.

We concluded that this indirect (noninvasive) method is applicable for estimating the actual exposure of the neonate during lactation.

Although estimates of daily milk ingestion appear to be valid for control rats, it is necessary to consider what effects the presence of lead might have on milk quality, quantity, or utilization by the neonate. As an indirect indication of the status of these parameters, food and fluid intake by the dam and growth rate of the neonates have been closely monitored. The effects of lead acetate in lactating dams' drinking water were examined over a range of concentrations from 0.02 percent to 2.0 percent. We found that alteration in food and fluid intake occurred at all concentrations above 0.2 percent. At these same concentrations depression in neonatal growth rate was evident. However, at a concentration of 0.2 percent lead acetate, all of the above parameters were unaltered. Therefore, it would appear that the presence of lead at the concentration used in this study does not significantly alter the validity of the assumptions underlying the theoretical estimates of daily lead intake.

Employing this methodology we have estimated the daily exposure of the Pentschew and Garro (1966) model wherein neonatal rats suckle dams producing 40 ppm lead-containing milk from birth to 21 days of age. The pups then wean to the dam's diet which contains 31,000 ppm lead. This protocol is commonly used as an animal model of lead encephalopathy. Prior to weaning, the exposure of the neonate is about 1.5 μg Pb/g/day (Figure 4-1, upper solid curve). Following weaning, daily lead exposure jumps to 3,000 μg Pb/g/day, a 2,000-fold increase.

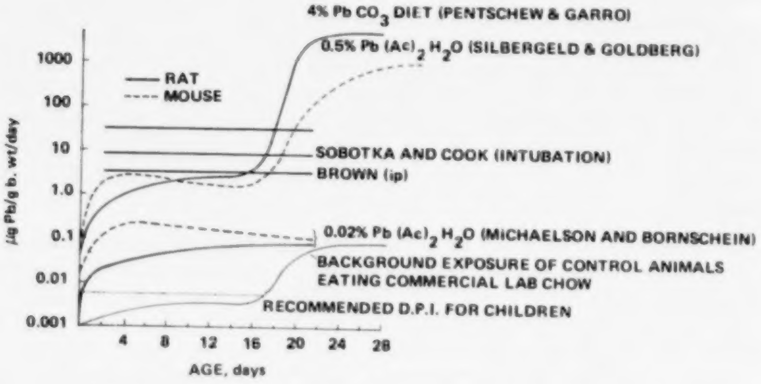

Figure 4-1. Daily lead exposure levels in rats and mice during pre- and postweaning periods.

These estimates indicate that neonatal rats suckling a non-lead-exposed dam (milk lead <0.01 ppm) have a daily lead exposure of 3 μg lead per kg per day (Figure 4-1, lowest solid line). Note that lead exposure increases upon weaning onto normal lab chow since commercial chows are lead contaminated (Fox *et al.*, 1976). The "hyperactive" mouse model described by Silbergeld and Goldberg (1973, 1974) is illustrated by the upper broken line of Figure 4-1 and demonstrates the similarity in exposure levels to that found in rats when employing Pentschew and Garro's (1966) procedure. These levels are unacceptably high for studies of low-level lead exposure.

This procedure for estimating daily exposure level is also of value in permitting comparisons between this method of exposure and injection or intubation procedures. Brown (1975) and Sobotka *et al.* (1975) have utilized injection and intubation techniques to administer lead directly to neonatal rats. These investigators have reported both behavioral and biochemical changes following neonatal exposures ranging from 5 to 50 μg Pb/g body wt/day. These exposure levels are in excess of that obtained during the suckling period with the Pentschew model. However, the resultant blood lead concentrations are considerably lower. For example, Sobotka *et al.* (1975) report that daily intubation of 45 μg Pb/g body wt results in blood lead concentrations of 71 μg percent. We have found that presenting the lactating dam with water containing 0.2

percent lead acetate results in an estimated exposure of the neonate to about 0.5 μg Pb/g body wt/day, resulting in a blood lead concentration of about 65 μg percent. The difference in resultant blood lead concentrations is most likely a reflection of higher absorption levels obtained with the milk vehicle (Kostial *et al.*, 1971) and of a higher body burden due to a more nearly continuous daily exposure to lead.

Using these estimation techniques, it is also possible to compare exposure levels in rats and mice with those seen in the human population. Recommendations pertaining to maximum daily permissible intake D.P.I. for various segments of the human population range from 4 to 10 μg Pb/kg body weight (Barltrop, 1973). Most investigators using the lactating dam as a vehicle for lead administration are exposing the neonates to lead levels 10 to 100 times higher than the recommended daily permissible intake for the human population. The ability to make such comparisons, i.e., between various exposure techniques and between animal models and the human population, should enhance our ability to develop relevant animal models of lead exposure for the human pediatric population.

Our investigations of biochemical and behavioral effects of lead exposure are being carried out in animals receiving lead at a level approximately one order of magnitude greater than the level recommended for children (see Figure 4-1). The exposure level or external dose is of course only one of several important descriptive indices. Perhaps of greater importance is the internal dose as reflected by blood lead concentrations and brain lead concentrations. We have undertaken an analysis of the transfer of lead from the dam's water to the blood and brain of the neonate. A summary of the results appears in Table 4-3, which reveals that the blood lead levels span those concentrations of greatest interest, i.e., 20 to 80 μg percent.

Table 4-3. LEAD CONCENTRATION IN RAT BLOOD AND BRAIN AFTER 20 DAYS OF LACTATION[a]

Concentration of Lead acetate in dam's water	Dam's blood lead (μg%)	Dam's milk lead (μg%)	Pup's blood lead (μg%)	Pup's brain lead (μg%)
Tap water	9 ± 3	<1	11 ± 4	12 ± 2
0.02%	24 ± 5	21 ± 2	29 ± 5	29 ± 7
0.10%	41 ± 7	87 ± 7	42 ± 4	47 ± 10
0.20%	69 ± 21	139 ± 11	65 ± 25	65 ± 6
0.50%	104 ± 2	264 ± 40	137 ± 7	136 ± 31

[a] Values represent $\bar{X}$ ± SEM.

We have also assessed blood and brain lead levels at various points following termination of exposure at day 20. Tables 4-4 and 4-5 summarize the effects seen in rats and mice, respectively. There are several points of interest in Table 4-5. First, irrespective of the treatment group, i.e., acute or chronic exposure, the blood lead concentrations were highest at the end of the lactation period which is probably a reflection of the high absorptive capacity of the neonate. In the acutely exposed group, blood lead concentrations declined but did not reach control values. The chronically exposed group tended to reach steady state blood lead concentrations of about 120-140 μg percent. A second interesting finding is that brain lead levels in the acutely exposed group slowly decreased following termination of exposure. In the chronically exposed group, lead continued to accumulate in brain tissue despite a relatively steady-state blood lead. The potential pitfalls in any attempts to relate a specific biochemical or behavioral effect to a single determination of blood lead concentration at a single point in time are readily apparent.

Table 4-4. LEAD CONCENTRATION IN RAT BLOOD AND BRAIN
AGE: 20, 60, and 270 DAYS

Concentration of lead acetate	Blood lead concentration (μg%)		
	20	60	270
Tap water	11 ± 4[a]	4 ± 1	9 ± 0.3
0.02%	29 ± 5	5 ± 0	9 ± 0.7
0.1%	42 ± 4	9 ± 3	9 ± 1.0
	Brain lead concentration (μg%)		
Tap water	12 ± 2	14 ± 0	12 ± 1
0.02%	29 ± 7	8 ± 0	14 ± 0.3
0.1%	47 ± 10	14 ± 0	20 ± 4

[a] N = 3 to 5 subject for each treatment x age group. Values represent $\overline{X} \pm$ SEM.

Table 4-5. LEAD CONCENTRATION[a] IN MICE AS A FUNCTION OF AGE AND TREATMENT

Age, days	Concentration of lead in dam's water					
	⟨0.05 ppm Pb		109 ppm Pb[b]		2780 ppm Pb[c]	
	Blood	Brain	Blood	Brain	Blood	Brain
15	--	--	42	16	--	--
20	15	5	79	24	190	200
40	17	5	29	24	120	306
100	4	4	20	10	143	584

[a] Pooled samples—4-5 mice per sample—reported as μg%.

[b] Exposure via maternal milk only – exposure terminated at day 21.

[c] Continuous exposure – during first 3 weeks via maternal milk and postweaning via dams water supply.

Lead Exposures and Nutritional Status

The effect of lead ingestion on the normal pattern of food and water intake by rodents has been well documented (Michaelson and Sauerhoff, 1974; Golter and Michaelson, 1975; Morrison *et al.*, 1975; Maker *et al.*, 1975). Furthermore, we have recently reported on the effects of developmental undernutrition on motor activity, a behavioral parameter also reported to be affected by lead exposure (Loch *et al.*, 1976). The results of these studies indicate the need to consider the dietary status of animals as a major variable in experimental design. Dietary factors can be regarded as both a previously uncontrolled variable which can confound the interpretation of experimental findings and as a potential independent variable which adds a critically important dimension to the development of an animal model for asymptomatic exposure.

In demonstrating the role of dietary status as an uncontrolled variable in most lead toxicity studies, it should first be pointed out that numerous dietary constituents have been shown to influence lead metabolism. The list is extensive and includes: protein and fat content; trace metals, e.g., iron, copper, and zinc; vitamins, e.g., vitamin D; and essential minerals, e.g., calcium and phosphate (Barltrop and Khoo, 1975).

Absolute nutrient concentrations as well as concentrations relative to other dietary components can drastically alter the toxic effects which may ensue following exposure to lead. Commercially available animal chows are characteristically oversupplemented with essential nutrients. Often they exceed by 5- to 10-fold the levels recommended by the National Research Council (1972) for normal growth and development. The concentration of any particular dietary constituent may vary considerably between different manufacturers as well as between different lots of chow obtained at different points in time from the same manufacturer. However, concentrations seldom fall below the NRC recommendations. The extent of variability between different suppliers and the extent of oversupplementation can be seen in Table 4-6. In this table are listed several dietary constituents known to influence lead metabolism. Recommendations of the NRC can be compared with dietary concentrations reported by several manufacturers. Table 4-7 illustrates the extent of the variability between advertised metal content and that actually found in various lots obtained over the course of 3 years. Note also the high concentrations of lead and cadmium in successive shipments from the same supplier. The obvious conclusion to be drawn from these results is that it is essential that investigators control, document, and report those aspects of the animal's diet which are known to influence lead metabolism. Until this is done, we will continue to encounter difficulties replicating work within our laboratories as well as that work carried out in other laboratories.

Table 4-6. NUTRIENT COMPOSITION OF COMMERCIAL RAT DIETS

Dietary Constituent	NRC Requirements	Supplier number			
		1	2	3	4
Fat (%)	7.6	9.0	6.0	7.0	10.0
Ca (%)	0.6	1.2	2.0	1.1	0.9
Cu (ppm)	5.6	18.0	13.0	15.5	6.5
Fe (ppm)	39.0	198.0	370.0	370.0	37.0
Zn (ppm)	13.0	58.0	38.0	--	20.0
Vit D (I.U./g)	1.11	3.3	5.1	2.4	4.0

Table 4-7. TRACE METAL AND HEAVY METAL CONTENT OF COMMERCIAL DIET NO. 1[a]

	Metal content (ppm)						
	Zn	Cu	Cr	Mn	Fe	Cd	Pb
NRC	13	6	--	56	39	--	--
Advertised	58	18	--	51	198	--	--
Sample 1 (1974)	53	11	1.4	60	278	0.1	3.0
2 (1975)	55	17	1.8	62	209	1.3	2.8
3 (1975)	60	17	2.1	60	465	0.8	3.1
4 (1975)	68	13	1.3	57	475	0.9	3.0
5 (1975)	58	13	2.4	53	415	1.1	2.5
6 (1976)	54	9	--	--	363	--	1.0

[a] Unpublished data from Dr. L. Murthy and H. Petering of the Department of Environmental Health, University of Cincinnati.

In addition to concerns pertaining to dietary status as a control variable, we also argue for its consideration as a significant independent variable in any attempt to model asymptomatic pediatric lead toxicity. As noted previously, rats consuming commercial lab chows are being subjected to a diet which is heavily fortified with essential nutrients. Such diets are seldom encountered in the human population. In fact, it has been well documented that a substantial portion of our population is deficient in one or more of the nutrients considered essential for normal health. The parameter of greatest concern with respect to the lead problem may be iron deficiency. The recent Ten State Nutritional Survey (1968-1970) indicates that in children under three years of age, 50 percent were deficient in iron intake irrespective of ethnic group or socio-economic status. Approximately 40 percent of this population had iron intakes less than one-half the Recommended Dietary Allowance. The findings are of considerable importance because of the demonstrated biochemical interactions of lead and iron in important biochemical pathways involving electron transport, catecholamine metabolism, and porphyrin synthesis. Similar arguments could be made for several other dietary constituents.

Iron deficiency alone can produce significant biochemical and behavioral changes, many of which bear a striking resemblance to the clinical picture obtained with elevated lead exposure (Pollit and Leibel, 1976). Dietary intake of other trace metals, e.g., zinc and copper, is also considered to be marginal in some segments of our population. Animal models of asymptomatic lead exposure should provide the capability for controlling and manipulating dietary status. Dietary manipulations in conjunction with various levels and durations of lead exposure can reveal much about the mechanisms of lead neurotoxicity as well as suggest possible methods for preventing or correcting lead's effects on critically sensitive segments of the population. For these reasons our animals are maintained on a semipurified diet which closely approximates the NRC recommendations.

During the 21-day period prior to weaning, the neonate's nutritional status is directly related to the amount of milk consumed per unit time per gram body weight and the concentration of essential nutrients in the dam's milk at that time. Utilizing the same methodology as that used to estimate daily lead intake, it is possible to estimate the daily intake of iron, copper, zinc, or other essential nutrients by the neonate at any point during the lactation period. For example, we have determined the zinc content of control dam's milk at 5-day intervals during lactation. These data, together with estimated daily milk consumption, permit us to estimate daily zinc intake during the lactation period. Table 4-8 illustrates the result of such a calculation. By employing such a procedure, it is possible to define the daily intake of critical nutrients such as iron, copper, zinc, and other metals known to interact with lead. It may also be possible to manipulate the dam's diet in order to produce specific trace metal deficiencies in the neonates. Such manipulations may permit an evaluation of lead exposure in conjunction with specific dietary deficiencies in the neonate.

Table 4-8. ESTIMATED DAILY ZINC INTAKE

Day of lactation	5	10	15	20
Pup body wt (g)	12	24	37	50
Zinc content of milk (ppm)	17	13	11	10
Milk ingestion (g)	2.8	7.4	12.8	18.2
Zinc intake (μg absolute)	47.6	96.2	140.1	182.0
Zinc intake (μg/g body wt)	4.0	4.0	3.8	3.6

Unintentional Sources of Exposure

As indicated in Table 4-6, commercial laboratory chows contain considerable concentrations of lead (Fox *et al.*, 1976). This concentration poses several problems in regard to the preparation of adequate control animals. Long-term consumption of these chows can result in an elevated lead body burden. During pregnancy, the fetus is subjected to unintentional and, at this point, unpreventable exposure to lead. Furthermore, at parturition the neonates are subjected to a substantial increase in lead body burden as a result of elevated lead concentration in dam's milk. Finally, beginning on about day 17 of life, the neonate begins to consume solid food, producing about a 15-fold elevation in daily lead exposure after weaning if the chow contains 0.5 ppm lead (see Figure 4-1). Since gastrointestinal absorption is still very high in the 17- to 30-day-old rat (Forbes and Reina, 1972), a marked elevation in body burden can occur. Of far greater consequence is the lead exposure which can result if the 15- to 20-day-old rat pup should gain access to the dam's lead-containing water supply. For example, a dam drinking 0.2 percent lead acetate in water produces milk containing about 1.4 ppm lead. An 18-day-old rat pup consuming this milk is exposed to about 0.5 μg lead/g body wt/day. If this pup should ingest one ml of the dam's water supply, its exposure is increased to 22 μg lead/g body wt/day. Fortunately, this type of unintended lead exposure can be prevented with the appropriate choice of cages. The greatest potential source of unintended lead exposure is that lead which is introduced into the neonate's environment by the dam. The lactating dam drinking 0.2 percent lead acetate-water consumes about 80 ml of water or about 87,200 μg of lead per day. The dam retains less than 5 percent of this total (Kostial *et al.*, 1971). The remainder, about 82,000 μg of lead, is excreted through the urine and feces into the neonate's immediate environment. If cage bedding is changed only every 5 days, lead contamination can increase to 410,000 μg of lead. How much of this lead is available for intake by the neonate is unknown. Most of this lead is in fecal material, and some is adsorbed onto the surface of the bedding material. Clean ground corn cob bedding contains 0.06 ppm lead. After 5 days, the bedding, free of fecal material, contains 36.0 ppm lead. The high level of licking, preening, and oral exploratory behavior in which neonatal rats normally engage can result in the unwanted transfer of some of this lead into the neonate. In experiments where bedding material was changed only once a week, we found that within 3 weeks the neonates had a lead body burden 4 times greater than would be predicted on the basis of lead intake via milk only.

In this paper we have attempted to highlight some of the major problems associated with the experimental exposure of neonatal animals to inorganic lead. It is our hope that appropriate attention to these problems will lead to a further refinement of current models of low-level chronic neonatal lead exposure. We further hope that our methodology for estimating daily exposure levels will generate new dialogue concerning the adequacy and relevance of animal models currently used to assess the effects of low-level lead exposure on the developing nervous system.

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5. OBSERVATIONAL TECHNIQUES IN BEHAVIORAL TOXICOLOGY

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Introduction

Observational techniques, at their simplest, require no more equipment than pencil and paper and a willingness on the part of an observer to describe as much of the behavior as interests him or as his sensitive, but sometimes unreliable, eyes and ears can capture. In this guise, observational techniques have had a long history of use by lovers of the natural history of animals, by field ecologists, and, more recently, by the school of ethologists, led into the field by Konrad Lorenz to capture the natural history of animals in the snares of theory, quantification, and laboratory experimentation. After generations of scientific indifference or, even more, disdain for the presumed anthropomorphic and subjective elements in all observation of animal behavior, the value of controlled observation of animals—freely expressing the subtleties of social, maternal, or even simple exploratory behavior—is now accepted and exploited as a way of investigating the function of the central nervous system. Table 5-1 covers the areas which have been most extensively investigated in recent years.

Table 5-1. BEHAVIORS STUDIES BY OBSERVATIONAL TECHNIQUES

Locomotor acts	Social behavior	Reproductive behavior	Early development	Rhythmic behavior
Exploration Hypoactivity Hyperactivity	Dominance roles Territoriality Communication	Mating Maternal	Sensory reflexes Motor function	Diurnal rhythms Circadian rhythms

Given that observational techniques have achieved acceptance as powerful quantitative methods of studying behavior, do these techniques have a useful role in the study of behavioral toxicology? The answer is an unequivocal "yes" for reasons to be explained.

It is a truism, or should be, that behavior is the output of the central nervous system. Behavior is produced by the CNS in response to various external and internal stimuli received from the external environment and the body. In addition, behavior is presumably produced by spontaneous activity of the CNS itself without direct stimuli, at least in higher animals. The sum of these activities of the CNS, when expressed as changes in motor function, becomes the output known as behavior. If behavior is defined as spatially directed, complex motor acts, the following are excluded: the amorphous phenomena known as emotions, volition, and consciousness; and specific nondirected muscle movements such as tremors, twitches, and seizures. In the study of toxicants which affect the CNS, the potential alterations in behavior are many. The effects of toxicants can range in duration from rapidly reversible, as in the case of therapeutic drugs which last for a few hours, to permanent changes, as in the effects of x-irradiation on the developing brain. Toxicants can directly affect sensory, motor, or integrating systems in the CNS and hence alter behavior. Behavior can also be altered when the CNS is not the target organ for the toxicant but is affected indirectly as a result of a toxic effect elsewhere in the body. Examples of indirect effects on behavior are numerous. Kidney and liver damage obviously can affect behavior as can cardiovascular effects. Altered levels of circulating hormones and damage to peripheral sensory and motor systems modify behavior. In fact, it is difficult to conceive of damage to the body anywhere which cannot affect behavior. Thus behavioral toxicology cannot be limited to specific kinds of effects, sites of action in the CNS, or to reversible phenomena, and in these three ways behavioral toxicology differs from behavioral pharmacology. These considerations bring us to the use of observational techniques in behavioral toxicology. If one is interested in whether or not the behavior of an animal has been modified in any way by exposure to a toxic substance, such a global question could require examination of a wide range of behaviors in order to get a reliable answer. Alternatively, one could look for a global answer by examining a few behaviors which reflect a generalized function of the CNS. The difficulty here is knowing which behaviors to study. This problem leads to the argument which favors observational techniques as methods for obtaining global answers. If behavior is the consequence of the processing and integration by the CNS of the three sources of information listed before (external and internal stimuli and spontaneous CNS activity), the easiest of these to control experimentally are the external stimuli. If the external stimuli are kept constant and if the external environment is as neutral as possible (i.e., if the animal is not pressured experimentally to perform any particular motor acts), then the remaining two factors predominate. Since these are the two factors which will be altered by toxic substances, it follows that observation of spontaneous behavior in a neutral environment should reflect changes in various CNS functions brought on by the toxicant. This logic suggests that observational techniques should be sensitive for detection of many types of toxicants. The next question concerns the reliability of observational techniques and the methods by which quantification of spontaneous behavior can be achieved.

The most common method for quantification of spontaneous behavior is to record the frequency with which an act occurs. Thus many studies have reported the number of incidents of given acts per unit time. An example of this method, drawn from pharmacology, is found in the study by Silverman (1965) of the effects of drugs on the frequency of various acts performed by rats before and after drug administration. In addition to frequency analysis, a more elaborate analysis will include the duration of behavior acts as well as frequency, introducing another concept. It is inherent in the use of frequency and duration in reference to behavior acts that such acts must be discrete quantities which can be defined in some way as starting and stopping and hence having a unique appearance in the stream of movement of an animal from birth to death. It has been argued that behavior acts are not "real," i.e., are not discrete, and that, therefore, quantitative analysis of behavior acts is not possible or, at least, is not reliable. This argument is equivalent to the criticism that observational tests produce highly "subjective" data. This point deserves careful consideration since the discrete nature of behavior is intuitively favored by most observers of animal behavior. The evidence for the concept has been discussed in detail previously (Norton, 1968) and will not be repeated here, but the ability to define specific behavior acts in terms accurately recognized by a computer from video film (Norton and Servoss, 1972) is a strong argument in favor of the nonsubjective nature of spontaneous behavior.

The consequence of the discrete nature of behavior acts is that observers of spontaneous behavior have at their disposal rich and varied sets of data which can be analyzed in many ways that have hardly been explored at the present time. In general terms, spontaneous behavior can be viewed as having both a macrostructure and a microstructure, depending on the time scale in which the analysis is carried out. Moderately long-term trends are apparent in diurnal and circadian rhythms associated with the light-dark cycle, and short patterns of behavior appear in the rapid cycling of acts in many situations, such as exploration of a novel environment. Figure 5-1 presents a simple illustration of these relationships. Is there any real difference in the macro-and microstructure of behavior other than the time frame? The answer to this question is not clear at present. The distinction is made here to point out the relationship between these areas of research, but there may also be a valid physiological distinction. For example the long-term behavioral sequences in the macrostructure, such as sleep-wakefulness, may be dependent on internal changes such as diurnal shifts in adrenal cortical hormones; whereas the microstructural changes are more dependent on rapid local modification of CNS activity which is responding to rapidly changing sensory stimuli in the environment. Generally, different techniques are used to record these two major divisions of behavioral structure. Because the macrostructure is concerned with slow behavioral processes, the usual forms of analysis are concerned with recording only on-off timing of the behavior, as in sleep-wakefulness cycles. Another common technique is to measure circadian alterations in activity, a technique exemplified by the use of photocells to monitor locomotion in a residential cage throughout the day (Barnett and McEwan, 1973; Norton *et al.*, 1975; Reiter and Ash, 1976).

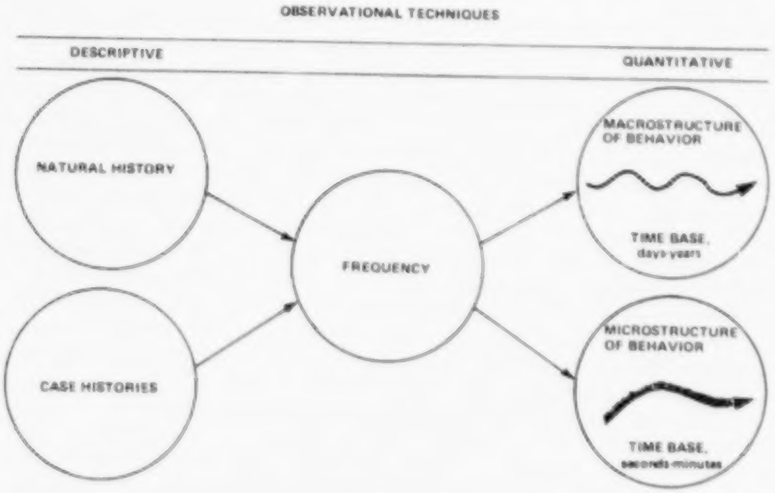

Figure 5-1. Schematic relationship of macro- and microstructure of behavior.

The most precise technique presently available for studying the microstructure of behavior is unquestionably to record the behavior on photographic film or video tape, a procedure which gives the observer a permanent record from which to study the behaviors of interest in any desired detail. The drawback to working with a permanent visual record is that it can contain a tremendous amount of information.

The first step in data reduction should take place before the permanent record is even made. That is, before the observer records the animal's behavior, he should select an environment appropriate to produce the desired behavior. As Suomi and Harlow (1969) pointed out several years ago, all cages in which animals are placed are instruments which affect the behavior. If it were possible, it would be ideal to have time stand still in the analysis of short bouts of behavior, but time is also a variable to the CNS, and the brain clearly responds to what has just transpired as well as to immediate events. In this way, the output of the CNS resembles a partial Markov chain in which each event has a relationship to the preceding event in the chain. An example of such a chain of behavioral events is shown in Figure 5-2. This figure depicts the type of behavior obtained when a rat is placed in an exploratory situation, for example, in a simple plastic box of the type shown in Figure 5-3, and is photographed with a time-lapse cinecamera at one frame per second. Placing the rat in the box triggers off a sequence of behavioral acts. These discrete acts average about 3 seconds in duration, and transition times between discrete acts are very short compared to the duration of the acts. This difference in duration and transition times allows the use of time lapse to reduce data acquisition without much loss of significant information.

MICROSTRUCTURE OF BEHAVIOR

PHOTOGRAPHIC ANALYSIS

RE	RE	RE	ST	ST	ST	ST	ST	SI	SI
SM	PW	PW	LO	LO	LO	HT	HT	WF	WF
1	2	3	4	5	6	7	8	9	10

TIME, seconds

Figure 5-2. Example of microstructure of behavior of rats in an exploratory situation as recorded by time-lapse photography at one frame per second. RE, rearing; SM, smelling; PW, pawing; ST, standing; LO, looking; HT, head turning; SI, sitting; WF, washing face.

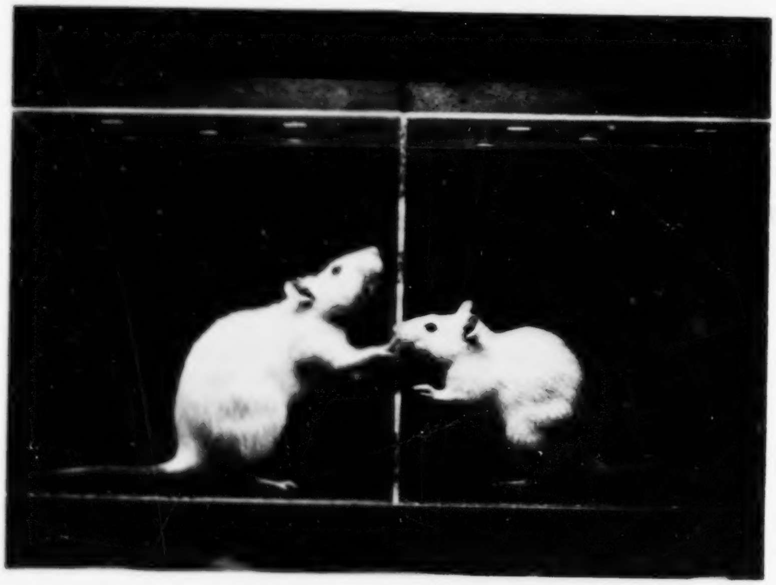

Figure 5-3. Photograph of chamber used for time-lapse analysis of exploratory behavior of rats.

Having decided upon the behaviors of interest (in the experiment described here it is the exploratory behavior of the rat) and the method of producing a permanent record of that behavior, three quantitative aspects of the behavior are obtained from the record: the durations, the frequencies, and the associations of behavioral acts. When pairs of rats are photographed at one frame per second for 15 minutes, the photographic record for each rat is 900 frames of film. In order to handle even this amount of data, computer programs are needed to analyze the results. The behavioral act of each rat in each frame of film is identified by the observer. The body position (standing, sitting, rearing, walking, turning, or lying down) and head or paw position (looking, smelling, head turning, sniffing, bobbing, washing face, grooming, scratching, or pawing) is identified for each second. These behavioral acts of rats have been defined previously (Norton, 1973) although in general the words follow conventional English usage.

The following computations are made from the data obtained on groups of rats (i.e., experimental and control groups):

1. The duration (D) in seconds of each act listed above is determined. Average durations and standard deviations are calculated for each act. In the data examined so far, the durations are normally distributed or have a gamma distribution.

2. The frequency (F) of each act is determined as the number of times each act is initiated, regardless of duration. Average frequency per rat and standard deviation are determined conventionally.

3. The total number of seconds that each act is performed is calculated per rat. This number is obviously the product, D x F.

4. Behavior patterns (behavior sequences) are estimated. The computations involved here will be described in some detail since this represents the least familiar (and most revealing) parameter measured.

Determination of Behavior Patterns

In the long history of observation of animal life, behavior patterns of animals have been described often. As an example, drawn from classical biology, many observers have described the patterns in mating behavior of various species. Mating patterns have been seen in some insects where the behavior is a deterministic chain in which each step follows a rigid form dependent on the previous act and from which there is no deviation. Even in vertebrates—for example, in some fish (Nelson, 1964; Barlow, 1968)—the pattern of mating behavior has a highly rigid structure. In most of these studies, the behavior pattern is so precisely executed that the pattern has been described without reference to alternate patterns or variability in the patterns. In other studies, such as those on verbal communication among rhesus monkeys by Altmann (1965), the inherent flexibility in sequences of acts is so great that probability measurements are required to identify patterns. It is not surprising that both situations have been described: that some behavior patterns are "hard-wired" so that some behaviors are performed in an unvarying sequence following the initiation of the sequence by one of a pair of animals, and, alternatively, that some behaviors are performed in sequences with a probability that is dependent on many variables including the preceding behavior. Then are there any random acts performed without dependence on preceding acts? There is no unequivocal answer to this question because the available data are not adequate. It can be predicted only on the basis of present data that there may not be any truly random behavior acts, at least not among the higher vertebrate groups.

In the case of behaviors which follow each other with sequential probabilities, i.e., those in which the behavior pattern is not completely determined, the strategy for detecting the pattern needs to be considered. This is particularly true of complex patterns which, to the unaided eye, may superficially appear to be random sequences. The most straightforward approach is to calculate probabilities directly for various sequences of acts in which the observer is interested. While this is an accurate and efficient method, it has two drawbacks. First, it requires that the sequence of interest be predicted by the observer. Second, it is limited to short sequences, i.e., three or four acts, because the calculations required to obtain absolute probabilities of all possible combinations and permutations of longer sequences are prohibitive.

An alternate strategy to calculate sequences for large numbers of acts is to compute chi square values for observed and expected frequencies of each pair of behavior acts as they are obtained in successive time bins in the photographic record. For example, if there are 14 behavior acts in 900 seconds of exploratory behavior of each rat, observed and expected occurrence of pairs of the 14 acts in successive 2- to 30-second intervals or longer can be readily determined. This method has been used to detect three patterns of exploratory behavior in rats under these circumstances (Norton, 1968; Norton, 1973). As an example of the data obtained with these calculations, Figure 5-4 contains the details for a pair of acts, walking and rearing. This figure shows that the number of simultaneous occurrences of walking and rearing is less than expected in intervals shorter than 5 seconds, while in intervals 5 to 30 seconds long, walking and rearing are paired more frequently than expected. The difference in observed and expected frequencies is significant in intervals from 9 to 20 seconds using a chi square test for goodness of fit of the observed to the expected occurrences. Since walking and rearing are acts which each last several seconds, once initiated, it is not surprising that they are found together with less than random expectation in intervals less than 5 seconds. The duration histograms in Figure 5-4 show the actual durations of each of these acts. The significant association of walking and rearing as a pair in the longer intervals up to 30 seconds shows that the initiation of one of the pair increases the probability of occurrence of the other act. If these calculations are continued for all possible pairs of the 14 behavior acts identified from film in these experiments, the most probable sequence of behavior can be determined. Table 5-2 shows this sequence for a group of 28 control rats. The interval used to determine the sequence can be any time bin from 5 to 20 seconds in these experiments with rat behavior, and the same sequence will be obtained.

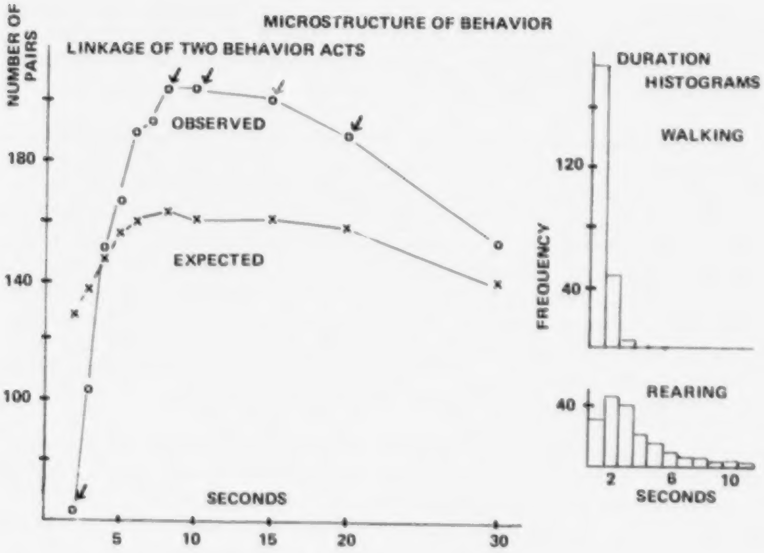

Figure 5-4. Example of nonrandom association of 2 behavior acts, walking and rearing, in exploratory behavior of controls rats. In line graph, X = expected occurrences of walking and rearing together, in time intervals from 2 to 30 sec, calculated from observed frequency of occurrence of each act independently; O = observed occurrences of walking and rearing in the same intervals. Arrow points to time intervals where difference between observed and expected was significant (p = <0.05, chi square test for goodness of fit).

Table 5-2. MICROSTRUCTURE OF BEHAVIOR SEQUENCE OF BEHAVIOR ACTS IN CONTROL RATS

(Abbreviations are defined in the text)

WF SI GR SC	BO WA TU RE PW SN	HT SM ST LO
Grooming	Exploratory	Attention

Table 5-3 shows the data from which a complete sequence of 14 acts is obtained, using the calculations of chi square values for observed and expected pairs. This table shows the chi squares for pairs of behavior acts in a group of control rats (upper right half of matrix) and a group of rats with lesions in the globus pallidus (lower left half of matrix). The linear arrangement of the acts is the same as that in the sequence of acts in Table 5-2 and is obtained by sorting the acts for control rats so that the diagonal adjacent to the main diagonal of the matrix reaches the maximum numerical value of the chi squares in which observed frequency is greater than expected. The effect of this arrangement is to put acts together which have the highest chi squares for the pairs occurring together and also to separate pairs with large chi squares which were less frequent than expected. The negative sign is used in Table 5-3 to indicate the latter chi squares. Only one such arrangement with the maximum numerical value for the summed diagonal is obtained for each matrix, and this arrangement determines the most probable behavior sequence. The labels given to three clusters of acts—grooming, exploratory, and attention—are for convenience in identifying the types of acts involved. The abbreviations for the acts are: WF, washing face; SI, sitting; GR, grooming; SC, scratching; BO, bobbing; WA, walking; TU, turning; RE, rearing; PW, pawing; SN, sniffing; HT, head turning; SM, smelling; ST, standing; and LO, looking.

Table 5-3. MATRIX OF CHI SQUARES FOR PAIRS OF BEHAVIOR ITEMS IN SUCCESSIVE 5 SEC INTERVALS IN 4 CONTROL AND 4 LESIONED RATS[a] ARRANGED TO MAXIMIZE NUMERICAL VALUE OF CONTROL DIAGONAL ADJACENT TO MAIN DIAGONAL (MINUS SIGN USED TO INDICATE OBSERVED BEHAVIOR WAS LESS FREQUENT THAN EXPECTED)

	Pairs of acts in control rats													
	WF	SI	GR	SC	BO	WA	TU	RE	PW	SN	HT	SM	ST	LO
	Grooming				Exploratory-Grooming						Attention-Grooming			
WF	--	45	15	2	−6	−16	−7	−5	−3	−8	−12	−39	−28	−5
SI	31	--	31	0	0	−9	−2	−2	4	0	−13	−39	−25	−3
GR	5	7	--	0	−2	−2	0	−5	0	−2	−1	−7	0	−1
SC	0	0	0	--	−2	−2	0	−5	0	−2	−1	−7	0	−1
	Grooming-Exploratory				Exploratory						Attention-Exploratory			
BO	−3	2	−1	0	--	0	2	5	2	3	0	0	2	1
WA	−5	−3	−1	0	0	--	1	0	−1	−3	1	2	2	0
TU	−7	0	−1	0	1	0	--	24	1	0	0	2	1	−1
RE	−8	0	−2	0	7	1	14	--	5	2	0	4	0	0
PW	−1	5	0	0	1	0	0	6	--	2	0	0	0	0
SN	0	0	0	0	0	0	0	0	0	--	0	0	0	2
	Grooming-Attention				Exploratory-Attention						Attention			
HT	−10	−5	−7	0	0	0	0	0	0	0	--	7	5	0
SM	−8	−12	−5	0	−5	0	0	1	−1	0	0	--	12	0
ST	−6	−4	−3	0	0	0	0	−1	0	0	2	0	--	4
LO	−7	0	−1	0	0	0	0	0	0	0	1	−8	2	--

[a] Control rat data in upper right half of matrix; lesioned rat data in lower left half of matrix.

In the experiment in Table 5-3, each lesioned rat was photographed with a control rat as shown in Figure 5-3. Although there is some effect of one rat on the other in these paired circumstances, the effect is to make the animals' behavior more alike, thus slightly reducing the difference between control and experimental animals (Norton, unpublished observations). The data in Table 5-3 show that the effect of the lesion was to reduce the chi square values in each cluster of acts. The interpretation of this phenomenon is that the lesion affected the microstructure of behavior, causing the rats to perform acts in a more random fashion. This experiment has been reported previously in greater detail (Norton, 1976).

The significance of the microstructure of behavior and the effect of toxic conditions on it need consideration. First, it appears that there is a structure to behavior even under conditions where an observer may not readily detect it. The example given here is the analysis of a simple exploratory situation in which a rat is placed in a plastic cage for 15 minutes. Under these conditions control rats show a distinct patterning of behavior. Furthermore, this patterning is disrupted by certain types of brain damage. In the case shown here, damage was caused by stereotaxic placement of bilateral lesions in the globus pallidus.

In an analysis of observed behavior, the frequency and duration of behavior acts should be examined as well as the sequences. Such an analysis of behavior should be able to detect toxic effects on behavior in experiments in which a less complete analysis might miss one or more of these alterations. Although it has not been directly demonstrated, it is possible that certain types of brain damage could affect the structure of spontaneous behavior and yet cause little or no effect on other behavioral parameters. If spontaneous behavior of man and other animals is observed in various naturally occurring and environmentally limited situations, and if the structure of the behavior is examined, it should be possible to widen the range of toxic effects which can be experimentally quantified. This ability may be valuable both in the detection of toxic behavioral effects and in understanding the ways in which the output of the central nervous system is organized.

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6. LOCOMOTOR ACTIVITY MEASUREMENTS IN BEHAVIORAL TOXICOLOGY: EFFECTS OF LEAD ADMINISTRATION ON RESIDENTIAL MAZE BEHAVIOR

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Introduction

Various measures of locomotor activity have been widely used in behavioral toxicology research. The popularity of this endpoint is probably due to the ease with which "some" measure of activity can be made. Several of the methods currently employed have been described by Finger (1972). Typically, these methods measure locomotor activity over short periods of time. Open field activity measurements, for example, range from 2-5 minutes in duration (Archer, 1973) and therefore, record what may be a unique activity, generally referred to as "exploratory activity." Although this is a useful measure, it does nothing to describe the animal's "Spontaneous" activity levels which require measurement in a more familiar environment. Further, chemically-induced changes in exploratory activity may differ from those recorded in a more familiar environment.

Biological rhythms are another factor which influences an animal's responsiveness to chemical agents. Reinberg and Halberg (1971) have introduced the field of chronopharmacology which is concerned with the effects of drugs (and toxins) as a function of biologic timing. It is not surprising that many classes of drugs, such as stimulants and depressants, show circadian rhythms of potency in various species including man. By limiting behavioral measurements, especially measurements of locomotor activity, to a single time of day (typically the diurnal period), one overlooks the possible interaction of biorhythms with the toxic effects of an agent.

Norton *et al.* (1975a) have recently described a residential maze system designed to measure locomotor activity in either grouped or isolated animals over extended periods of time. This system utilizes a number of photocells to record passage of an animal through a series of interconnected alleys (see Figure 6-1) and has been shown to be capable of detecting behavioral disruption by a variety of factors (Norton *et al.*, 1976; Reiter *et al.*, 1975). Our laboratory has implemented a modification of this system which allows for computer controlled data acquisition and analysis, thus making the maze particularly useful for long-term activity measurements.

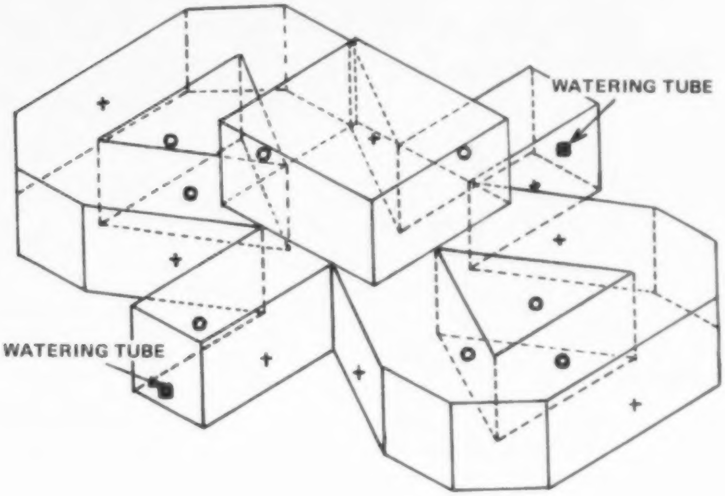

Figure 6-1. Schematic representation of the residential maze used to measure locomotor activity. The maze consists of a series of interconnected alleys (10 cm wide, 10 cm high) converging on a central open field and covered with transparent acrylic plastic equipped with a central raised area over the open field. The overall dimensions are 76 cm wide x 60 cm deep. Locomotion is detected by eight phototransistors (+)/infrared light-emitting diode (o) pairs (Reiter *et al.*, 1975).

The present paper describes a series of experiments which measured the effects of continuous pre- and/or postnatal exposure to lead on locomotor activity. Some short-term measures of activity were made in preweanling animals, but in general, activity was measured in adults (120 days old) over extended periods ranging from 5-14 days. In this way, we could study various components of an animal's activity, including exploratory, diurnal, and nocturnal activity, as well as ultradian and circadian rhythms. The results indicate that lead administration affects these components of activity in a dissimilar manner and serve to illustrate the utility of this approach.

Methods

Locomotor activity of adult males was recorded at approximately 120 days of age in the residential maze (Figure 6-1). Groups of three littermates were tested in the maze for a period of either 5 or 14 days. Four mazes were housed in a sound-attenuated room maintained on a 12-hour light/12-hour dark cycle beginning at 6:00 a.m. Food and water were available *ad libitum*. The daily experimental procedure was as follows: Groups were introduced into the mazes at 10:00 a.m. and removed at 9:00 a.m. the following day. The mazes were cleaned, food and water were replenished, and the animals were then reintroduced into the mazes at 10:00 a.m. Activity counts, indicating the number of times a rat passed a photodetector in an arm of the maze, were recorded hourly with the use of a laboratory minicomputer (Computer Automation, Inc.).

Measurements of residential maze activity were also obtained following subcutaneous administration of d-amphetamine, which the animals received 20 minutes prior to being reintroduced into the maze. In Experiment 1, we gave only one dose of amphetamine (4.0 mg/kg), which we administered on the fifth day in the maze. In Experiment 2, utilizing doses of 1.0, 2.0 and 4.0 mg/kg, a dose response to amphetamine was obtained. Animals receive each dose of the drug, with two days allowed for recovery between drug administrations. The sequence of drug administration was balanced both within and across treatment groups.

Social interaction was also measured in the residential mazes. This behavior was recorded on video tape, under dim red light, approximately 2 hours into the nocturnal period on the third day in the maze. Interaction was measured as the number of contacts and the total time in contact between any two of the three animals during a 10-minute observation period. A contact was defined simply as two animals physically touching and required a break of at least one second between contacts. Due to availability of equipment, only two groups per treatment group were recorded.

Male offspring in Experiment 2 were also tested for acute exploratory activity at various ages of development, both prior to and following weaning. This activity was measured in individual animals placed in a jiggle-cage (Lafayette Instrument Company, Model A 501) for a 4-minute test period at 13, 16, 29, and 44, days of age. This method measures movement of the animals on a springloaded platform and is, therefore, affected by the animal's body weight. Since there were marked differences in body weight between treatment groups, comparisons of jiggle-cage activity were possible only between lead-treated and pair-fed controls.

Experiment 1

Male and female Sprague-Dawley rats, obtained from Blue Spruce Farms (Altamont, N. Y.), were randomly assigned to one of three treatment groups: 0, 5, or 50 ppm lead administered in the drinking water in the acetate form. Purina lab chow was available *ad libitum*. Starting at 50-60 days of age, both males and females were pretreated for a period of 40 days, which allowed lead concentrations to approach steady-state levels in soft tissues (Castellino and Aloj, 1964). Following pretreatment, animals were mated, and pregnant females were continued on lead throughout gestation and lactation. At weaning, the offspring were similarly exposed through 180 days. These F_1 generation rats were mated at 90 days of age, and the resulting F_2 generation animals were exposed to the same lead regimen. No differences between F_1 and F_2 generations were observed in any parameter, and results for both generations have been combined.

Experiment 2

Timed-pregnant Sprague-Dawley rats, obtained from Charles River Breeding Laboratory (Willmington, Massachusetts), were used. The lead exposure regimen was similar to that of Sauerhoff and Michaelson (1973). Briefly, ground Purina lab chow containing 5 percent lead carbonate was provided to nursing mothers at parturition. Two control groups were employed: one group received food *ad libitum*, and a second group was pair-fed to lead-treated mothers. Pair feeding was accomplished by measuring daily food consumption in lead-treated mothers and providing a matched pair-fed mother with that amount of food on the following day. This pair-fed group served as a control for the decreased food consumption observed in the lead-treated animals. Lead was removed from the chow on the sixteenth day postpartum and added to the drinking water, as lead acetate, in a concentration of 50 ppm. At 21 days, offspring were weaned onto lead-containing water and continued on treatment throughout the experiment.

Results

The 23-hour activity of control male Blue Spruce Sprague-Dawley rats, taken on the fourth day in the maze, is shown in the upper portion of Figure 6-2. Following an initial period of exploratory activity, during the first hour after reintroducing the rats into the maze, the control animals showed a normal circadian rhythm with low diurnal activity and high nocturnal activity. The mean activity levels (counts/hour) obtained during these various periods, i.e., exploratory, diurnal, and nocturnal, as well as total activity, were used to test for treatment effects.

Figure 6-3 represents control data for the adult male Sprague-Dawley rats obtained from either Blue Spruce (Experiment 1) or Charles River (Experiment 2). Data are presented for the first four days in the maze during the time periods just described. Locomotor activity was higher on the first day than on subsequent days. This elevated activity represents the animal's increased reactivity due to the novel environment. In Blue Spruce animals, activity was elevated through the nocturnal period of the first day. Any activity measurements of 23 hours or less will therefore include some component of exploratory activity. Animals become "established" in the maze by the second day, showing consistent activity levels on subsequent days.

Although both control groups showed the same general pattern of activity in the maze, we observed some differences. First, Blue Spruce animals were more active than Charles River animals. On day 1, the Blue Spruce animals were

40 percent and 160 percent more active than Charles River animals during the nocturnal and diurnal periods, respectively. By day 4, this difference had diminished to 3 percent and 50 percent for the same periods. Second, activity of both groups fell on day 2, but this fall in activity was greater in Blue Spruce animals, such that the ratio of day 1/day 2 activities was greater in the Blue Spruce. The ratio of day 1/day 2 activity for the diurnal period was 3.5 for Blue Spruce and 2.0 for Charles River rats. This elevated ratio indicates that Blue Spruce animals were not only more active, but were also different in their reactivity, showing a greater percentage drop in activity as the novelty of the environment wore off.

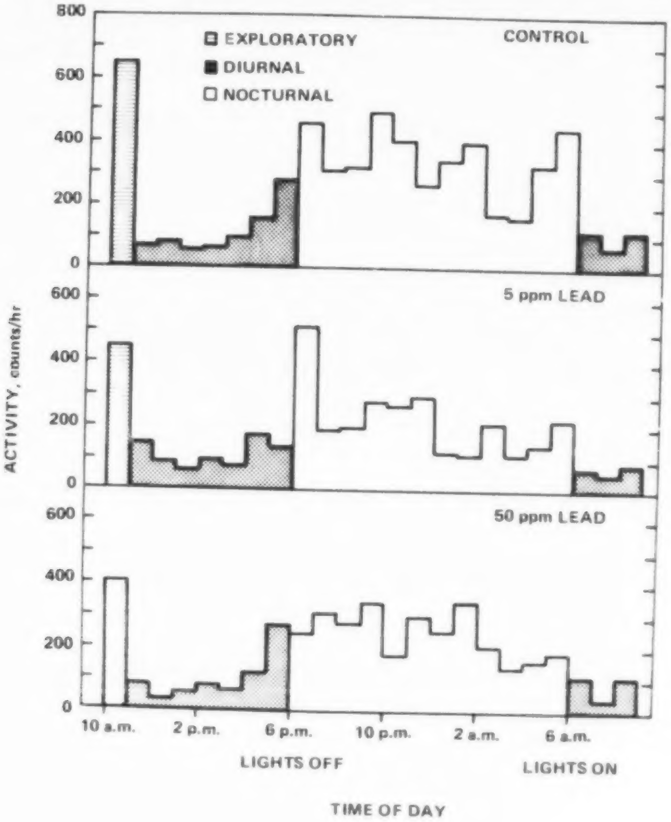

Figure 6-2. Representative time-interval histograms from control and lead-treated animals (Experiment 1). Data for each histogram represent means for 5 groups of adult males (3 animals/group) obtained on day 4 in the maze. For purposes of analysis, the data has been divided into various activity periods represented by the shaded areas.

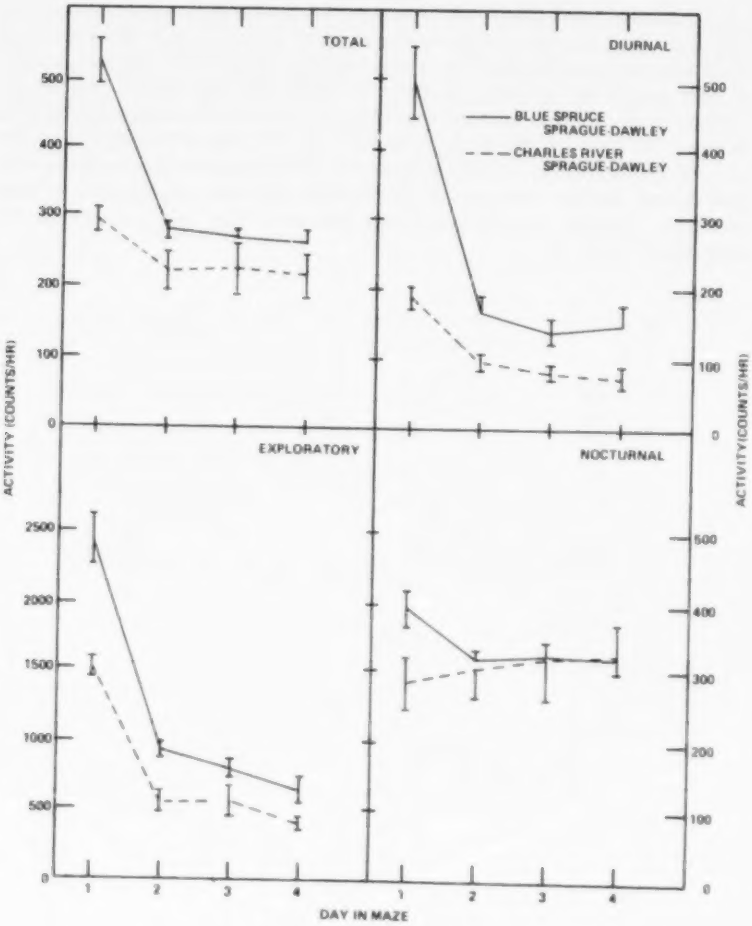

Figure 6-3. Control locomotor activity for adult male Sprague-Dawley rats obtained from two suppliers. Animals were tested in groups of 3, and each point represents the mean counts/hr (± SE) for either 4 (Charles River) or 5 (Blue Spruce) groups of animals. Data are presented for the various activity periods for the first 4 days in the maze.

Experiment 1

The residential maze activity levels (expressed as mean counts/hour) of the various treatment groups for the first four successive days of testing appear in Figure 6-4. Lead administration resulted in a significant depression of locomotor activity which ranged from 27-42 percent on day 1. This lead-induced hypoactivity was not dose-related although tissue lead levels demonstrated a dose-related internal exposure (Cahill *et al.*, 1976). Both the total and the nocturnal activity remained depressed throughout the 4-day period. By day 4, neither lead-treated group differed from control during the exploratory period, and following the first day, no treatment differences were found in the diurnal period. Therefore, the treatment differences seen in total activity are primarily a reflection of the depressed activity during the nocturnal period.

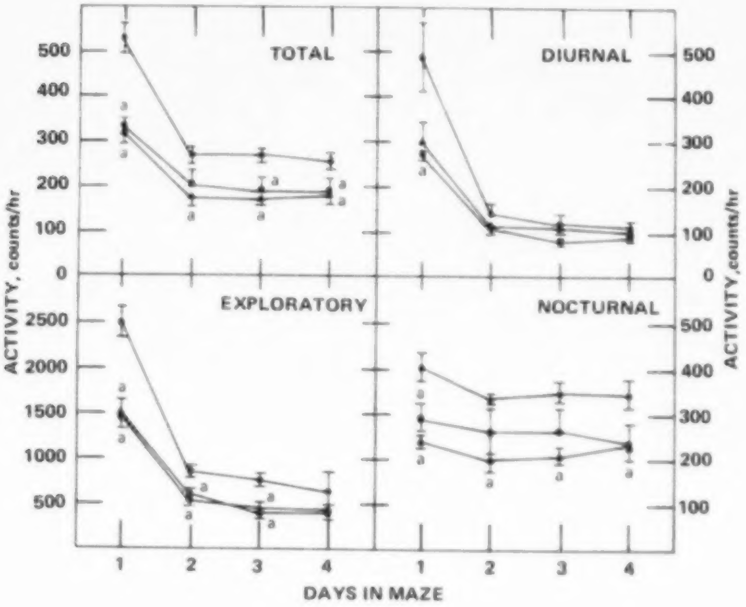

Figure 6-4. Effects of lead administration on locomotor activity in adult male rats (Experiment 1): (●) control; (▲) 5 ppm Pb; (■) 50 ppm Pb. Animals were tested in groups of 3 and each point represents the mean (counts/hr ± SE) for 5 groups of animals. Data are presented for various activity periods for the first 4 days in the maze. Points marked with "a" are significantly different from control (t-test), $p < 0.05$ (Reiter *et al.*, 1975).

The social interaction measured on the third night in the maze, approximately two hours into the nocturnal period, is represented in Figure 6-5 as the total number of contacts between any two of the three animals as well as total time in contact during a 10-minute observation period. Although these data represent only two groups of animals per treatment and were not subjected to statistical analysis, they suggest that social interaction is increased with lead treatment. These differences are especially striking for the total number of contacts, where the range of values for lead-treated groups does not overlap the control range.

Finally, locomotor activity was measured following 4.0 mg/kg d-amphetamine (sc) given 20 minutes prior to maze testing on day 5. Figure 6-6 presents the results as change in activity (Δ) following amphetamine administration. The Δ activity was determined by subtracting the activity obtained on day 4 (predrug, control levels) from activity following amphetamine administration and therefore represents the drug-induced change in activity.

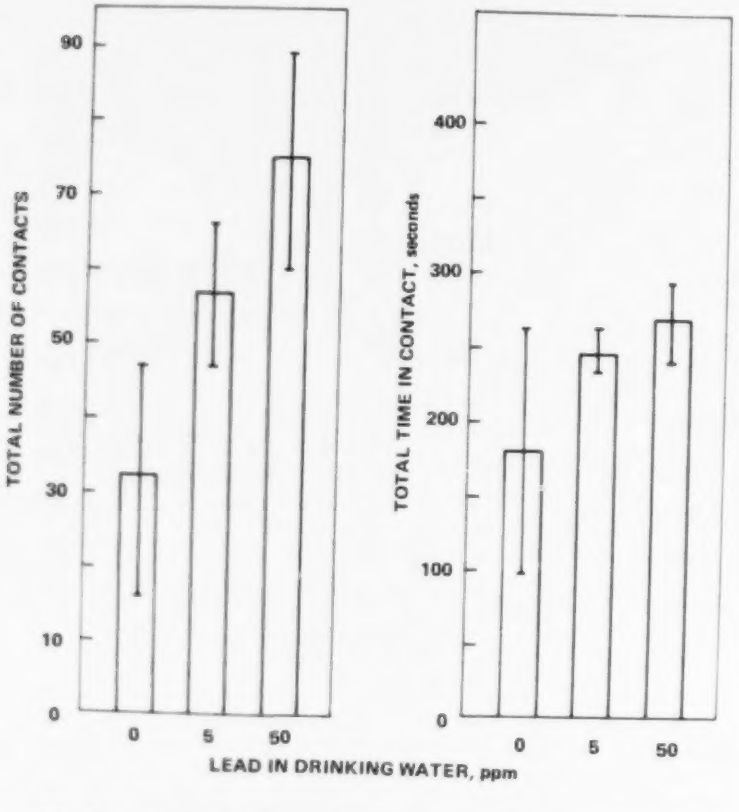

Figure 6-5. Effects of lead administration on social behavior in the adult male rat. (Experiment 1). Animals were scored for number of contacts and total time in contact in the residential maze during a 10-minute period of nocturnal activity on day 3 in the maze. Vertical bars represent the range of values.

In control animals, amphetamine produced the expected hyperactivity. Since the drug was administered during the diurnal period, when activity is normally low, the major effect appeared during this time. Activity in control animals was elevated 444 percent and 682 percent of predrug levels during the exploratory and diurnal periods, respectively. These increases were reflected in an elevation of total activity to 224 percent of predrug control level. During the nocturnal period, the control activity fell to 80 percent of the predrug level, demonstrating a partial compensation for the drug-induced hyperactivity.

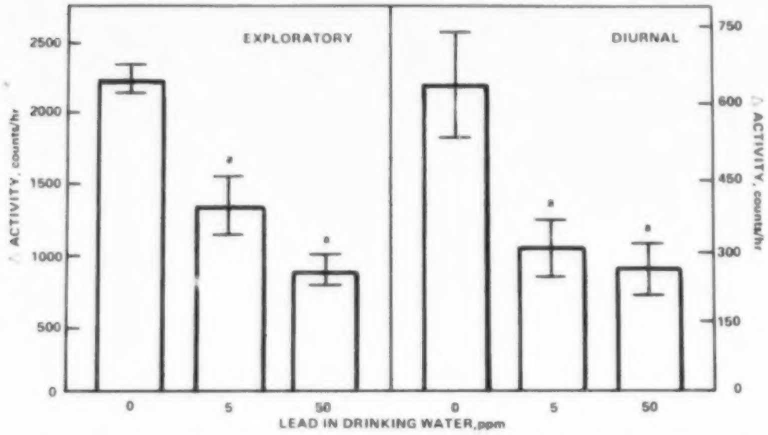

Figure 6-6. Effects of d-amphetamine administration (4.0 mg/kg sc) on locomotor activity of control and lead-treated rats (Experiment 1). Values are presented as Δ-activity (counts/hr ± SE) following amphetamine; Δ = (activity on drug day) - (activity on predrug day). Each bar, therefore, represents the drug-induced locomotor activity for 5 groups of animals. Bars marked with "a" are significantly different from control (t-test, $p < 0.05$).

Lead-treated animals showed a diminution in the amphetamine response (Figure 6-6). When compared to the Δ activity of the control group, both treatment groups showed a significant ($p < 0.05$) decrease in amphetamine-induced activity. This decreased responsiveness was also apparent when activity was expressed as percentage of predrug levels. Whereas control activity was elevated to 444 and 682 percent during the exploratory and diurnal periods, respectively, the 50 ppm group was elevated 323 and 298 percent during these same periods. As with the controls, treatment groups showed less activity during the nocturnal period, but no significant differences between treatment groups were present during this period.

Experiment 2

The acute exploratory activity of male offspring from mothers receiving 5 percent lead carbonate at parturition was measured in a jiggle cage at various ages. Figure 6-7 shows the results of these measurements, expressed as percent of pair-fed controls at these ages. Lead exposure produced a transient hyperactivity (208 percent of control at 13 days of age) which continued through 29 days of age and returned to normal by 44 days of age.

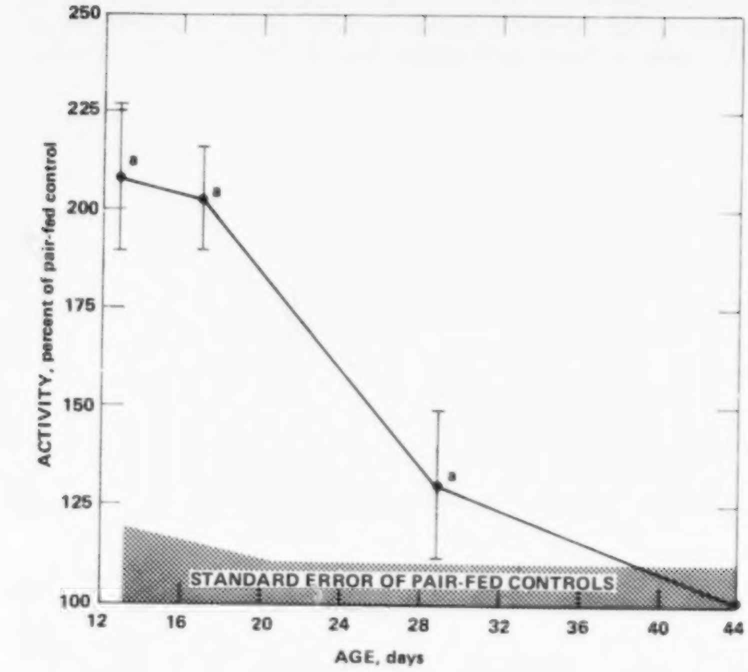

Figure 6-7. Effects of lead administration on acute exploratory activity in rats (Experiment 2), measured at various ages. Values are expressed as percentage of pair-fed controls. Each point represents the mean activity (± SE) for 5 litters (2 animals/litter). Points marked with "a" are significantly different from control (t-test, $p < 0.05$) Reiter, 1977).

Adult animals tested on the first 4 successive days in the maze showed no treatment effects relative to the total counts per 23 hours (Figures 6-8 and 6-9). However, there were notable treatment differences in activity following various doses of d-amphetamine, findings consistent with those of Experiment 1. Figure 6-10 shows the dose-response curves of d-amphetamine for the treatment groups. Again, the data are presented as Δ-activity and therefore represent drug-induced changes in locomotor activity. The three treatment groups responded differently to d-amphetamine administration. In general, the amphetamine response of treatment groups fell in the following order: pair-fed > *ad libitum* controls > lead-treated. Analysis of variance indicated a significant treatment-drug interaction ($p < 0.002$) during the diurnal period. Comparison of the lead-treated groups with pair-fed controls showed significant differences (t-test; $p < 0.01$) at the 2 and 4 mg/kg doses, during the exploratory and diurnal periods. Similar comparisons with *ad libitum* controls were significantly different only at the 4 mg/kg dose. It appears then that early undernutrition (pair-fed group) facilitates the response to amphetamine, shifting the dose-response curve to the left; whereas lead treatment attenuates the response, shifting the dose-response curve to the right. Since undernutrition is present in the lead group, a comparison between pair-fed and lead-treated groups is probably the more meaningful one.

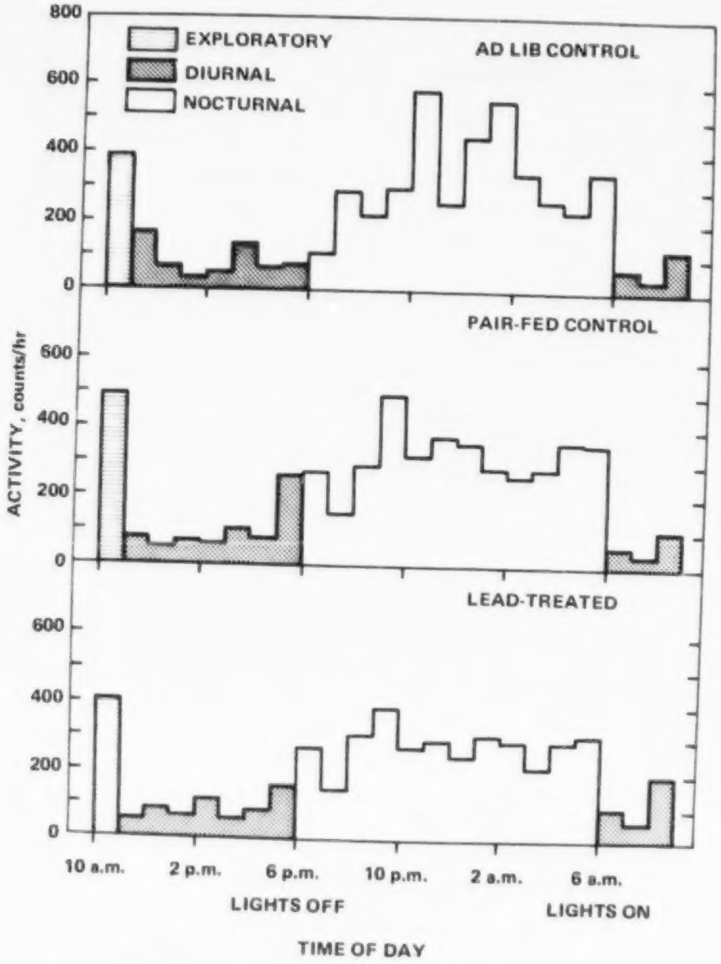

Figure 6-8. Representative time-interval histograms for control, pair-fed control and lead-treated animals (Experiment 2). Data for each histogram represent means for 4 groups of adult males (3 animals/group) obtained on day 4 in the maze.

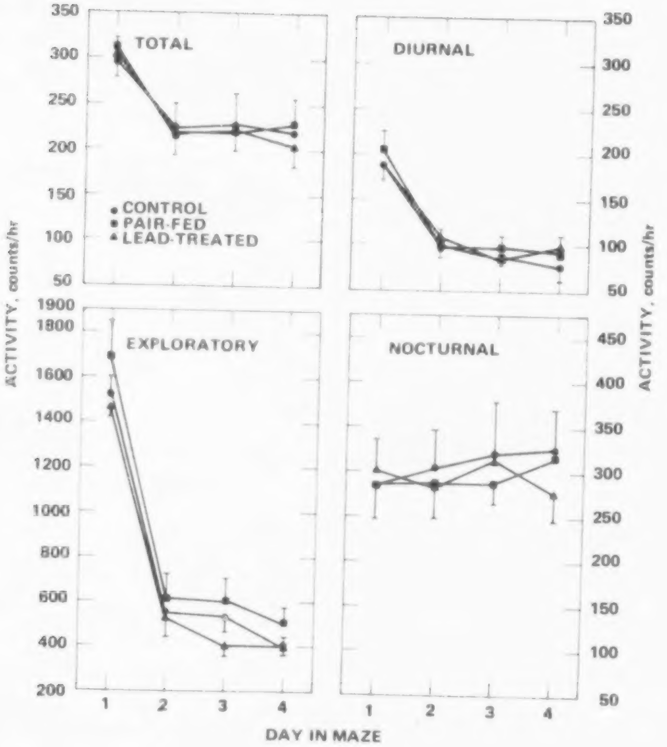

Figure 6-9. Residential maze activity of control and lead-treated adult male rats, tested in groups of 3 (Experiment 2). Each point represents the mean activity (count/hr ± SE) for four groups of animals taken on the first 4 days in the maze. No treatment differences were observed (Reiter, 1977).

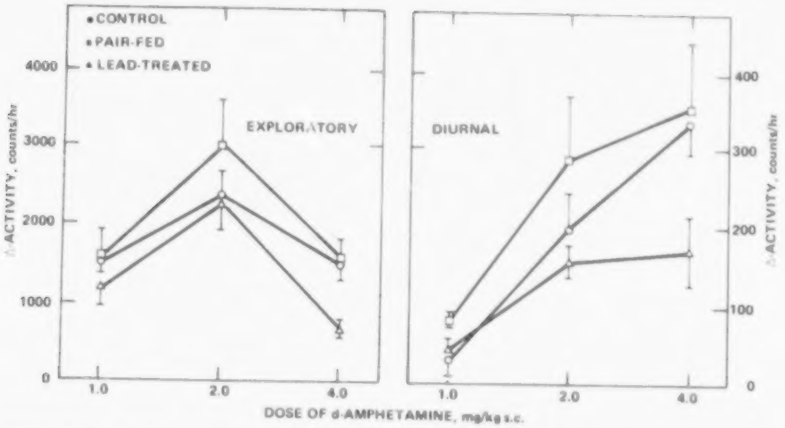

Figure 6-10. Dose-response curves for d-amphetamine in control and lead-treated animals (Experiment 2). Values are presented as Δ activity (counts/hr ± SE) for 4 groups of animals (Δ-activity calculated as in Figure 6-6). An analysis of variance indicated a significant drug-treatment interaction during the diurnal period ($p < 0.002$) with pair-fed animals showing a facilitated drug response and lead-treated animals showing an attenuated response.

During the nocturnal period, activity levels showed an apparent ultradian rhythm having an approximate 3-4 hour cycle (Figure 6-8). This rhythm was present in the control groups, partially disrupted in pair-fed controls, and completely disrupted in the lead-treated animals. Figure 6-11 shows predicted curves obtained for the various treatment groups pooled over three predrug days (days 7, 10, and 13 in the maze). Log_{10} transformations of the data have been performed to correct for heterogeneity of variance. Control animals showed a clear triphasic distribution of activity during the nocturnal period, with peaks occurring at 4-hour intervals. An analysis of variance indicated a highly significant effect of time ($p < 0.0001$) in these control animals, which accounted for 71 percent of the variability in the activity levels. Pair-fed controls showed some disruption in the later peaks. Nevertheless, an analysis of variance showed a significant effect of time ($p < 0.03$) in this group, with time accounting for 27 percent of the variability. Finally, activity in lead-treated animals showed no effect of time ($p = 0.88$), accounting for only 9 percent of the variability in this group. As previously indicated, we observed no treatment effects on activity levels during the nocturnal period, but there was a significant treatment x time interaction ($p = 0.05$), demonstrating that the nocturnal activity curves were not parallel. A further comparison of control with lead-treated animals, using an analysis of variance, indicated a significant treatment x time interaction ($p < 0.02$), thus demonstrating a difference between the predicted curves of these two groups.

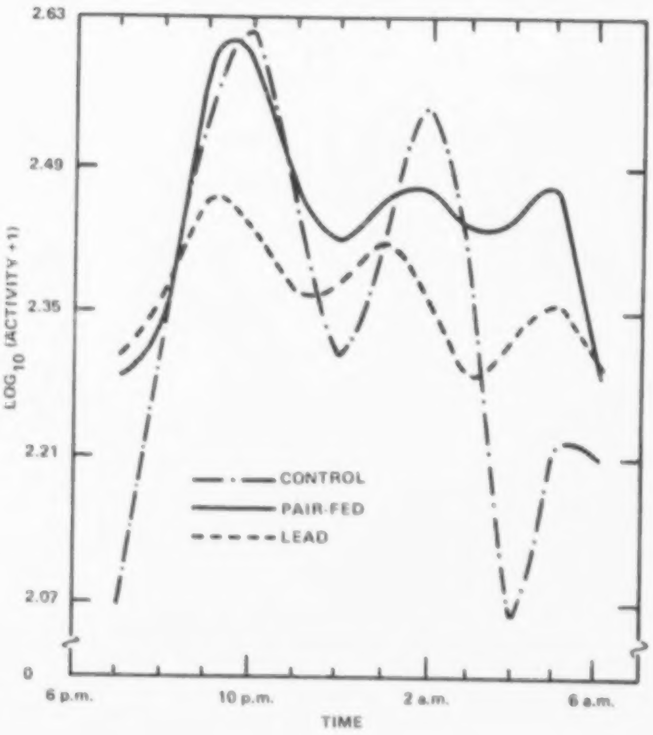

Figure 6-11. Predicted nocturnal activity curves for control and lead-treated adult male rats, tested in groups of 3 (Experiment 2). Log$_{10}$ transformations have been performed to correct for heterogeneity of variance. An analysis of variance indicates an overall treatment x time interaction ($p < 0.05$) demonstrating that the curves are nonparallel.

Discussion

In the previous chapter of this book, Dr. Norton separated spontaneous behavior into two categories, microstructure and macrostructure, based on the time scale in which the behavioral analysis is carried out. In the present study, the residential mazes serve as a useful tool in measuring the macrostructure of activity since a continuous recording of the animal's activity is obtained over extended periods of time. The present study observed several interesting facets of this macrostructure, and although the measurements were limited primarily to activity in the residential mazes, they are likely to occur to some extent in environments of different complexity.

There has been a tendency in the area of behavioral toxicology to use data taken from short-term measurements of ambulation to make general statements about locomotor activity with the assumption that a short-term measurement of activity will be representative of the macrostructure of activity in general. The data presented here do not support that assumption. The different facets of activity—i.e., exploratory, diurnal, and nocturnal—as well as the rhythms, appear to be different in both normal animals and animals exposed to lead.

Exploratory activity is operationally defined here as the ambulation occurring in a novel environment which exceeds the animal's baseline level of activity once established in that environment. As defined, exploratory activity in the residential maze extended well into the first day and, in the case of Blue Spruce animals, into the nocturnal period. Charles River animals, on the other hand, did not show elevated activity during the day-1 nocturnal period, indicating a difference in the duration of exploratory behavior in these animals. The two groups also differed in the relative degrees to which they responded with elevated activity on day 1. Whereas Blue Spruce animals were 350 percent more active on day 1 than on day 2, Charles River animals showed only a 200 percent elevation during the same period. Therefore, both the amplitude and duration of exploratory behavior were greater in the Blue Spruce animals.

Removing animals from the maze for one hour per day repeatedly produced an "exploratory burst" of activity when the animals were reintroduced into the maze. In both experiments, activity was elevated to approximately 500 percent of the normal diurnal activity level (see Figure 6-9). Therefore, daily one-hour activity measurements taken on consecutive days, although stable, will still represent exploratory behavior.

In addition to the familiar circadian rhythm in activity levels, the Sprague-Dawley rat also shows an ultradian rhythm. This rhythm, which has a 4-hour cycle, was pronounced during the nocturnal period. A similar pattern of resential maze activity was reported by Norton *et al.* (1975b) in adult female Sprague-Dawley rats obtained from Charles River. Although the data on ultradian rhythm were not presented for the Blue Spruce animals (Experiment 1), a similar pattern of activity was observed (see Figure 6-2). The apparent absence of ultradian rhythm during the diurnal period may be related to the experimental design. Since diurnal activity level is very low, it may be difficult to detect any small fluctuations, but more importantly, removal of the animals from the maze for one hour per day during the diurnal period may disrupt the rhythm. However, by the nocturnal period the animals have readjusted and the rhythm returns.

In Experiment 1, lead exposure produced changes in locomotor activity which differed during the various time periods. Exploratory activity was initially depressed in lead-treated animals, but this difference disappeared by the fourth day in the maze. Also, the treatment effect observed on day-1 diurnal activity probably reflects a change in the exploratory component of this activity which is present on the first day. On subsequent days, when exploratory behavior is no longer a part of the diurnal activity, the treatment effects disappear. The final treatment difference in activity levels, observed throughout the 4-day period, was the depressed nocturnal activity. It seems likely, therefore, that the depressed total activity is a reflection of this difference. A selective disruption of nocturnal activity in the rat has also been reported by Norton (1976) following lesions of the globus pallidus. In her experiments, residential maze activity was increased approximately 200 percent during the nocturnal period.

The lack of a lead effect on adult locomotor activity levels in Experiment 2 may have been due to the choice of the animal supplier (Charles River). Since Charles River animals are normally less active in the maze, it may be difficult to lower their activity further with treatment. This reasoning, however, is speculative, and certainly other differences in experimental protocol (i.e., dose and period of exposure) may account for these differences in observed activity between the two experiments.

Although there was no treatment effect on activity levels *per se*, lead exposure in Experiment 2 did result in a disruption of the nocturnal ultradian rhythm. However, this disruption cannot be attributed solely to lead treatment, since some change in the rhythm was observed in the pair-fed (undernourished) animals. Nevertheless, these results demonstrate that an alteration in the biological activity rhythm can occur with no accompanying alteration in the overall activity level. This finding presents an interesting situation. If one were to sample activity from rats in an established environment at 10 p.m. (4 hours into the nocturnal period), he would observe hypoactivity. Sampling at midnight would yield no difference in the activity levels. Finally, if measurements were made at 5 a.m., it would appear that treatment had produced a hyperactivity (see Figure 6-11).

The hyperactivity observed in young animals exposed to lead (Experiment 2) agrees well with the findings of Sauerhoff and Michaelson (1973) who reported increased activity in 28-day-old animals. Sauerhoff (1974) also found that lead-induced hyperactivity disappeared as the animals matured. This transient increase in activity may represent a delay in the animal's normal development. Some reports indicate that during the first 3 weeks of life, rats undergo normal developmental changes in activity with the peak level of activity occurring between 15 and 20 days of age (Campbell *et al.*, 1969). Since lead exposure has been shown to delay normal brain development in the rat, as indicated by delayed reflex development (Reiter *et al.*, 1975; Reiter and Ash, 1976), the possibility exists that lead is simply delaying this normal developmental pattern of activity.

In both experiments, the locomotor response to d-amphetamine was attenuated in lead-treated animals. This attenuated drug response has been previously reported in mice (Silbergeld and Goldberg, 1974) and rats (Sobatka and Cook, 1974). Furthermore, this diminished responsiveness to amphetamine has been observed when baseline activity levels of lead-treated animals were elevated (Silbergeld and Goldberg, 1974), normal (Sobatka and Cook, 1974; Experiment 2 of the present study), or depressed (Experiment 1 of the present study). This dissociation between the attenuated amphetamine response and the baseline level of activity in lead-treated animals would suggest that different neural systems are involved in these two responses. The work of Creese and Inversen (1975) has demonstrated that the integrity of the nigro-striatal pathway is essential for the locomotor response to amphetamine. It has also been suggested that a cholinergic link is involved in the regulation of the nigro-striatal dopaminergic pathway (Bartholini *et al.*, 1975). Since lead exposure has been reported to affect both the aminergic (Sauerhoff and Michaelson, 1973; Golter and Michaelson, 1975; Silbergeld and Goldberg, 1975) and cholinergic (Modak *et al.*, 1975) systems in the brain, it is possible that lead is affecting the nigro-striatal pathway resulting in an altered amphetamine response. However, until such an effect can be demonstrated directly, we must also entertain the possibility that lead treatment is altering the pharmacodynamics of amphetamine. By modifying the distribution and/or metabolism of amphetamine, lead treatment could be producing an attenuated response irrespective of a CNS effect.

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7. ALTERED BEHAVIORAL PATTERNING IN RATS POSTNATALLY EXPOSED TO LEAD: THE USE OF TIME-LAPSE PHOTOGRAPHIC ANALYSIS

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Introduction

Researchers commonly measure changes in levels of spontaneous locomotor activity to assess effects of CNS toxicants, drugs, and specific brain lesions on behavior. Most methods measuring activity, however, are restricted to quantifying changes in frequency, or possibly even duration, of a few select behaviors. This restriction allows the all-too-common possibility of finding an animal hyperactive with one method but not with another. For example, rats chronically exposed to low levels of lead were found hyperactive in a Y-maze and in a tilt-box, but not in a running wheel (Kostas *et al.*, 1976). Here, a toxicant's qualitative effect on locomotor activity depends upon the method of measurement; the method's capabilities, rather than the animals' behavior are dictating the results. This is a recurrent problem in behavioral toxicology, even when parameters such as habituation, presence of other animals, and timing during the light-dark cycle are kept constant.

Perhaps in a situation where a toxicant's effect on locomotor activity is inconsistent between methods, all possible parameters comprising changes in activity should be measured. Norton (1973) has postulated that changes in activity may consist of changes in sequential patterning as well as changes in frequency and duration of behavioral acts. All three—patterning, frequency, and duration—were altered in hyperactivities induced by amphetamine (Norton, 1973),

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globus pallidus lesions, carbon monoxide, and x-irradiation (Norton *et al.*, 1976). However, hyperactivity induced by morphine consisted only of changes in frequency and duration of behavioral acts (Norton, personal communication). Theoretically, it is possible that any one, either patterning, frequency, or duration, could be altered independently of the other two. Distinguishing changes in the patterning, frequency, and duration of many behavioral acts would certainly define alterations in locomotor activity more accurately and perhaps result in more consistent findings.

The purpose of this study is to measure the frequencies, durations, and patterning of behaviors in rats exposed postnatally to lead and their responses to the CNS stimulant, amphetamine. Exposure of rats postnatally to lead apparently produces an inconsistent effect on spontaneous locomotor activity; both hyperactivity (Sauerhoff and Michaelson, 1973) and no change in activity (Sobotka and Cook, 1974; Grant *et al.*, 1976) have been reported. As indicated in the study by Kostas and coworkers (1976), the method selected to monitor general activity in lead-treated rats may itself contribute to inconsistent findings. Perhaps the measurements proposed in this study can further understanding of the possible role a method of measurement may play in the inconsistent effect of lead upon spontaneous locomotor activity.

Methods

Charles River female rats (Sprague-Dawley derived proven breeders, 300-350 g) were mated with proven breeder males of the same strain. At parturition all litters were reduced to eight pups, and 5 mg/ml lead acetate were dissolved in boiled distilled water which was supplied *ad libitum* as drinking water to one group of mothers. This method of lead administration is routinely used at this concentration to produce hyperactivity in mice (Silbergeld and Goldberg, 1973, 1974, 1975). Drinking water containing lead was replaced with regular tap water after the first 10 days of the pup's suckling period. Restricting administration of lead-treated drinking water to the first 10 days after parturition was an attempt to minimize the effect of lead on body weights of suckling pups and yet still expose the pups during their critical period of sensitivity to lead (Brown, 1975). The other group of mothers received tap water during this period. At 28 days of age, all pups were weaned, separated according to sex, and housed in small groups in standard animal facilities. All rats received Purina Rat Chow and water *ad libitum*.

The animals were tested when they were 4 or 6 weeks, or 4 or 5 months old. At each age their body weights were recorded. A total of 22 groups of lead-treated and 22 groups of controls were studied, including 6 groups of lead-exposed and 6 groups of controls at each age, except at 4 months when only 4 lead-exposed and 4 control groups were tested. The groups, each consisting of 4 rats of one sex from a single litter, were independent and randomly selected at each age. An equal number of male and female groups were studied.

Frequency, duration, and patterning of animals' behaviors were measured using time-lapse photography. Experimental procedure was similar to that described by Norton (1973). One lead-exposed and one control rat were placed in opposite sides of a plexiglas cage, which was divided by a clear center panel. The rats were then filmed simultaneously as they explored the novel environment. They were filmed at a rate of one frame per second for the first 15 minutes they were in the cage. All rats filmed were identified by number only, so that each animal's behavior was analyzed without reference to its treatment. Researchers viewed films of the animals and recorded one of the following body positions of the rat for each frame: standing (ST), walking (WA), sitting (SI), rearing (RE), or lying down (LD). Furthermore, if present, one of the following behaviors was also recorded for each frame: scratching (SC), grooming (GR), washing face (WF), head bobbing (BO), turning (TU), pawing (PW), sniffing (SN), head turning (HT), smelling (SM), or looking (LO). Norton (1973) has previously described these behavioral acts in detail, and good inter- and intra-observer reliability for recognizing each individual act has been reported (Norton *et al.,* 1976).

Computer programs, similar to those used in the study by Norton and coworkers (1976), facilitated determinations of frequency, duration, and patterning of the various behaviors. The frequency of each behavior was determined from the total number of frames (or seconds) the behavior was observed during the 15-minute film of each rat. Average duration of each behavior was determined from the average number of consecutive frames (or seconds) that a behavior continued, once it was initiated. Frequency and average duration of each behavioral act were compared in lead-exposed and control rats using the t-test.

Patterning of behaviors in control and lead-exposed rats was compared by first determining the number of intervals where any two different behaviors occurred within 5 seconds of each other. This determination was accomplished by dividing the 15-minute film of each rat into 5 consecutive second intervals, then recording the number of 5-second intervals where two different, specified behaviors occurred. This determination was combined for all rats in a group. The occurrence of two different behaviors within 5 seconds of each other was defined as a "linkage" or "pairing" of those behaviors. For example, in the first 5 seconds of a film, if the behavior, rearing, occurred and if the behavior, pawing, also occurred at any time within the first five seconds, then one incident of rearing linked with pawing was recorded. This scoring was continued for remaining 5-second intervals and for all possible pairs of behaviors. In this study, since 15 different behaviors were scored, n(n-1)/2 or 105 pairs of two different behaviors were possible.

Observed occurrences of paired acts within 5-second intervals were compared with expected occurrences, and the chi-square value (goodness-of-fit) was obtained for each pair for all groups of rats. A matrix of so determined chi-square values for control groups was set up with the rows and columns arranged to obtain the maximum numerical value in the diagonal adjacent to the main diagonal of the matrix. This optimal arrangement was obtained in order to determine the most probable or preferred sequence of behaviors in control rats. The optimal sequence for control rats in this study was the same as that previously reported for controls (Norton *et al.,* 1976): LD-WF-SI-GR-SC-BO-WA-TU-RE-PW-SN-HT-SM-ST-LO. To determine if lead-exposed rats linked behaviors in this sequence as frequently as did controls, the observed frequencies of each pair of acts in this sequence in lead-exposed groups were averaged and compared with those for corresponding control groups using the Wilcoxon signed rank test. This comparison is referred to in the following results as the "main diagonal" comparison.

From the matrix of chi-square values, it was observed (Norton *et al.*, 1976) that within the optimal sequence of behaviors, there were three clusters of closely associated acts, based upon the high chi-square values for association of certain pairs. These clusters were labeled grooming, exploratory, and attention clusters. The grooming cluster included the behaviors lying down, washing face, sitting, grooming, and scratching. The exploratory cluster included head bobbing, walking, turning, rearing, pawing, and sniffing; the attention cluster included head turning, smelling, standing, and looking. In this study, the average frequency or number of 5-second intervals where each act was linked with other acts in the same cluster was compared in lead-exposed and control groups using the Wilcoxon signed rank test. These results are presented as comparisons of paired behaviors within clusters (grooming, exploratory, and attention). Furthermore, using the same statistical test, the average frequency or number of 5-second intervals where each act of one cluster was linked to each act in another cluster was also compared in lead-exposed and control groups. These results are presented as comparisons of paired behaviors between clusters (grooming linked with exploratory, grooming linked with attention, and exploratory linked with attention).

At six weeks of age, control and lead-exposed rats were filmed before receiving amphetamine and again the next day after receiving a subcutaneous injection of 0.5 mg/kg d-amphetamine hydrochloride in normal saline. The injections were given 30 minutes prior to filming so that during the time the animals were filmed, between 30 and 45 minutes after injection, the maximum effect of amphetamine at this dose would be observed (Norton, 1973). The frequency, duration, and patterning of behaviors after amphetamine were compared in control and lead-exposed rats as previously described.

Results

Average body weight of lead-exposed rats was not significantly lower than controls at any age their behavior was tested (Table 7-1). In general, weight differences between control and lead-exposed rats were insignificant, except for the slightly heavier weight of 6-week-old lead-exposed females.

Significant differences in frequency and duration (using the *t*-test) and in patterning (using the Wilcoxon signed rank test) of behaviors were observed between male and female rats at all ages, whether they were control or lead-exposed. Such differences precluded combining male and female data. However, since this study was not specifically designed to delineate sex differences, further studies must be conducted to determine if these differences were real.

Frequency of Behavior Acts

Frequency (total seconds of occurrence) of most behavior acts in lead-exposed animals, both males (Table 7-2) and females (Table 7-3), did not differ significantly from that in controls, whether they were 4 or 6 weeks or 4 or 5 months of age.

Table 7-1. AVERAGE BODY WEIGHTS (g ± SE)

Sex	Age	Controls		Lead-treated	
		No. of animals	Average weight	No. of animals	Average weight
Males	4 wks	35	76 ± 2	33	76 ± 3
	6 wks	22	195 ± 2	19	193 ± 4
	4 mo	8	498 ± 8	8	508 ± 9
	5 mo	17	577 ± 9	19	575 ± 9
Females	4 wks	20	73 ± 3	30	71 ± 2
	6 wks	16	147 ± 4	21	157 ± 3[a]
	4 mo	8	282 ± 10	8	295 ± 10
	5 mo	16	301 ± 8	18	316 ± 6

[a] Significant difference between lead-treated and controls (t-test, p 〈 0.05).

Table 7-2. TOTAL OCCURRENCES OF BEHAVIORAL ACTS IN MALE RATS (Av ± SE)[a]

Behaviors	Age			
	4 Weeks	6 Weeks	4 Months	5 Months
Controls				
LD	0	13 ± 9	68 ± 22	77 ± 44
SC	6 ± 2	4 ± 2	2 ± 2	4 ± 3
GR	69 ± 15	102 ± 12	95 ± 15	112 ± 23
SI	165 ± 30	168 ± 13	200 ± 29	195 ± 37
WF	86 ± 14	62 ± 5	93 ± 24	69 ± 10
BO	15 ± 2	8 ± 1	8 ± 1	8 ± 2
WA	33 ± 3	45 ± 5	12 ± 3	8 ± 1
TU	44 ± 3	38 ± 4	21 ± 4	15 ± 2
RE	72 ± 9	117 ± 14	111 ± 20	89 ± 13
PW	16 ± 3	19 ± 3	26 ± 9	25 ± 5
SN	19 ± 3	46 ± 7	23 ± 6	21 ± 7
HT	88 ± 7	85 ± 5	65 ± 8	49 ± 4
SM	224 ± 19	205 ± 14	183 ± 22	130 ± 12
ST	638 ± 35	557 ± 21	509 ± 27	531 ± 39
LO	96 ± 11	91 ± 8	86 ± 11	99 ± 11
Lead-treated				
LD	0	39 ± 18	52 ± 28	81 ± 25
SC	1 ± 1	1 ± 0	1 ± 1	1 ± 1
GR	32 ± 4[b]	57 ± 9[b]	112 ± 23	105 ± 23
SI	112 ± 10	133 ± 15	200 ± 31	196 ± 34
WF	75 ± 9	72 ± 7	82 ± 12	74 ± 9
BO	11 ± 2	8 ± 1	6 ± 1	10 ± 1
WA	35 ± 4	32 ± 5	13 ± 2	9 ± 3
TU	43 ± 3	32 ± 4	21 ± 4	18 ± 3
RE	87 ± 15	96 ± 13	109 ± 15	81 ± 20
PW	15 ± 3	16 ± 6	22 ± 5	17 ± 6
SN	30 ± 6	40 ± 9	51 ± 17	31 ± 8
HT	89 ± 9	95 ± 6	63 ± 5	51 ± 3
SM	276 ± 16[b]	193 ± 16	212 ± 12	165 ± 15
ST	667 ± 14	600 ± 22	527 ± 38	534 ± 41
LO	100 ± 11	80 ± 7	69 ± 12	57 ± 5[b]

[a] Control N = 12 and lead-treated N = 12 per age group, except control N = 8 and lead-treated N = 8 at 4 months.

[b] Significant difference between lead-treated and control groups (t-test, p 〈 0.05).

Table 7-3. TOTAL OCCURRENCES OF BEHAVIORAL ACTS IN FEMALE RATS (Av ± SE)[a]

Behaviors	Age 4 Weeks	 6 Weeks	 4 Months	 5 Months
Controls				
LD	0	2 ± 1	37 ± 29	10 ± 5
SC	5 ± 1	5 ± 3	5 ± 3	5 ± 2
GR	42 ± 7	54 ± 11	83 ± 21	64 ± 17
SI	97 ± 11	107 ± 16	150 ± 32	124 ± 23
WF	56 ± 6	52 ± 6	62 ± 12	49 ± 6
BO	11 ± 2	9 ± 2	6 ± 2	5 ± 1
WA	46 ± 4	55 ± 7	27 ± 2	28 ± 2
TU	51 ± 4	45 ± 6	24 ± 3	31 ± 2
RE	84 ± 10	122 ± 14	186 ± 41	244 ± 22
PW	15 ± 3	23 ± 3	16 ± 3	30 ± 4
SN	38 ± 5	52 ± 8	24 ± 3	29 ± 6
HT	104 ± 7	87 ± 7	63 ± 6	60 ± 4
SM	254 ± 16	256 ± 21	268 ± 16	282 ± 12
ST	673 ± 16	614 ± 29	504 ± 45	495 ± 24
LO	96 ± 9	81 ± 11	100 ± 16	118 ± 12
Lead-treated				
LD	0	5 ± 5	19 ± 15	31 ± 18
SC	5 ± 1	7 ± 3	1 ± 1	6 ± 3
GR	44 ± 7	73 ± 13	69 ± 14	84 ± 14
SI	131 ± 13[b]	140 ± 20	141 ± 25	155 ± 21
WF	77 ± 10	66 ± 8	62 ± 11	56 ± 8
BO	8 ± 2	9 ± 2	4 ± 1	5 ± 1
WA	43 ± 5	42 ± 4	26 ± 2	26 ± 3
TU	46 ± 3	44 ± 5	32 ± 2[b]	31 ± 3
RE	88 ± 14	102 ± 8	185 ± 19	223 ± 27
PW	16 ± 3	13 ± 2[b]	30 ± 5[b]	35 ± 6
SN	40 ± 5	47 ± 9	50 ± 13	34 ± 9
HT	89 ± 8	83 ± 6	66 ± 6	55 ± 4
SM	258 ± 22	240 ± 21	318 ± 20	282 ± 19
ST	638 ± 18	612 ± 14	528 ± 24	465 ± 25
LO	102 ± 12	95 ± 8	93 ± 9	87 ± 9[b]

[a] Control N = 12 and lead-treated N = 12 per age group, except control N = 8 and lead-treated N = 8 at 4 months.

[b] Significant difference between lead-treated and control groups (t-test, $p < 0.05$).

Duration of Behavior Acts

For all ages at which the animals were tested, average durations of most behaviors in lead-exposed males (Table 7-4) and females Table 7-5) were not significantly different from those in male and female controls respectively.

Patterning of Behavior

Patterning of behavior in lead-exposed groups was significantly different from that in controls. At all ages, significantly fewer occurrences of the pairing of different behaviors within 5-second intervals were observed in lead-exposed males (Table 7-6). This difference was maximum in 6-week-old animals. At that age, there were significantly fewer occurrences (1) of behaviors being linked in the optimal sequence characteristic of controls, (2) of each exploratory behavior being linked with other exploratory behaviors, (3) of attention behaviors being linked with other attention behaviors, and (4) of exploratory behaviors being linked with attention behaviors within 5-second intervals. Fewer occurrences of paired exploratory behaviors were still observed at 4 months, and decreased pairing of behaviors within the grooming cluster was also observed in 4-week- and 5-month-old lead-exposed males.

Table 7-4. DURATIONS OF BEHAVIORAL ACTS IN MALE RATS (Av Sec/Rat ± SE)[a]

	Age			
Behaviors	4 Weeks	6 Weeks	4 Months	5 Months
Controls				
LD	0	8.9 ± 6.9	27.7 ± 8.0	43.3 ± 35.3
SC	2.3 ± 0.7	1.6 ± 0.5	1.0 ± 0.5	1.0 ± 0.3
GR	3.9 ± 0.4	6.3 ± 0.6	7.3 ± 0.9	7.5 ± 0.9
SI	12.4 ± 1.9	18.3 ± 1.7	19.8 ± 2.1	23.7 ± 3.7
WF	5.5 ± 0.5	4.7 ± 0.4	6.7 ± 0.9	6.0 ± 1.0
BO	1.0 ± 0.01	1.0 ± 0	1.0 ± 0.01	1.1 ± 0.04
WA	1.2 ± 0.02	1.2 ± 0.03	1.2 ± 0.1	1.2 ± 0.1
TU	1.1 ± 0.02	1.1 ± 0.02	1.2 ± 0.03	1.1 ± 0.01
RE	2.1 ± 0.1	3.0 ± 0.2	5.6 ± 0.5	5.0 ± 0.5
PW	1.3 ± 0.1	1.6 ± 0.1	2.2 ± 0.2	2.3 ± 0.2
SN	1.5 ± 0.2	2.0 ± 0.2	1.8 ± 0.3	2.2 ± 0.3
HT	1.2 ± 0.01	1.1 ± 0.01	1.1 ± 0.02	1.1 ± 0.02
SM	2.1 ± 0.1	2.0 ± 0.04	2.4 ± 0.2	2.5 ± 0.2
ST	10.2 ± 1.6	7.9 ± 0.8	14.4 ± 2.4	22.7 ± 5.8
LO	1.4 ± 0.04	1.4 ± 0.04	1.7 ± 0.1	2.5 ± 0.4
Lead-treated				
LD	0	17.0 ± 8.7	14.8 ± 8.1	19.0 ± 5.0
SC	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.4	1.0 ± 0.2
GR	3.1 ± 0.5	4.5 ± 0.5[b]	10.1 ± 0.8[b]	9.7 ± 1.2
SI	10.2 ± 1.3	14.7 ± 1.4	25.2 ± 4.5	28.2 ± 4.6
WF	5.3 ± 0.4	5.5 ± 0.4	8.3 ± 1.5	8.8 ± 0.8[b]
BO	1.1 ± 0.03	1.0 ± 0.02	1.0 ± 0	1.1 ± 0.03
WA	1.2 ± 0.04	1.2 ± 0.02	1.2 ± 0.1	1.0 ± 0.1
TU	1.0 ± 0.01[b]	1.1 ± 0.02	1.1 ± 0.03	1.1 ± 0.02
RE	2.4 ± 0.1	2.7 ± 0.1	5.3 ± 0.5	4.8 ± 0.4
PW	1.3 ± 0.1	1.5 ± 0.2	1.9 ± 0.2	1.7 ± 0.2
SN	1.6 ± 0.1	2.0 ± 0.2	3.0 ± 0.4[b]	2.3 ± 0.3
HT	1.1 ± 0.02	1.1 ± 0.02	1.1 ± 0.02	1.1 ± 0.02
SM	2.4 ± 0.1	2.0 ± 0.1	2.6 ± 0.1	2.5 ± 0.1
ST	10.3 ± 1.0	10.4 ± 1.1	14.9 ± 2.2	21.7 ± 5.0
LO	1.5 ± 0.05	1.4 ± 0.05	1.5 ± 0.1	1.6 ± 0.1[b]

[a] Control N = 12 and lead-treated N = 12 per age group, except control N = 8 and lead-treated N = 8 at 4 months.

[b] Significant difference between lead-treated and control groups (t-test, $p < 0.05$).

Table 7-5. DURATIONS OF BEHAVIORAL ACTS IN FEMALE RATS
(Av Sec/Rat ± SE)[a]

Behaviors	Age			
	4 Weeks	6 Weeks	4 Months	5 Months
Controls				
LD	0	1.8 ± 1.5	11.1 ± 8.6	8.3 ± 4.8
SC	1.8 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	2.5 ± 0.9
GR	3.0 ± 0.4	3.6 ± 0.5	6.4 ± 0.9	5.7 ± 0.8
SI	8.1 ± 1.1	15.4 ± 2.6	19.4 ± 3.5	13.9 ± 2.1
WF	4.3 ± 0.3	6.6 ± 1.8	5.9 ± 1.0	4.7 ± 0.3
BO	1.0 ± 0.01	1.0 ± 0.1	1.0 ± 0.02	1.0 ± 0.02
WA	1.2 ± 0.02	1.2 ± 0.02	1.2 ± 0.03	1.1 ± 0.02
TU	1.1 ± 0.01	1.1 ± 0.02	1.1 ± 0.03	1.1 ± 0.02
RE	2.4 ± 0.1	3.0 ± 0.1	5.0 ± 0.5	5.7 ± 0.4
PW	1.3 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	2.0 ± 0.2
SN	1.7 ± 0.1	1.9 ± 0.2	2.0 ± 0.1	2.3 ± 0.3
HT	1.2 ± 0.03	1.1 ± 0.02	1.1 ± 0.03	1.1 ± 0.01
SM	2.1 ± 0.1	2.2 ± 0.1	2.8 ± 0.1	2.7 ± 0.1
ST	9.2 ± 1.0	11.4 ± 4.3	10.4 ± 2.5	7.7 ± 0.5
LO	1.4 ± 0.04	1.4 ± 0.05	1.6 ± 0.1	1.7 ± 0.1
Lead-treated				
LD	0	5.2 ± 4.6	10.2 ± 7.6	9.0 ± 4.3
SC	2.1 ± 0.5	1.3 ± 0.3	1.0 ± 0.3	1.0 ± 0.4
GR	3.4 ± 0.4	4.9 ± 0.5	7.4 ± 1.3	6.3 ± 0.6
SI	10.3 ± 1.4	13.0 ± 1.7	19.0 ± 4.9	18.3 ± 3.2
WF	4.9 ± 0.7	4.8 ± 0.5	4.6 ± 0.4	4.9 ± 0.4
BO	1.0 ± 0.1	1.0 ± 0.02	1.0 ± 0.04	1.0 ± 0.02
WA	1.2 ± 0.03	1.2 ± 0.02	1.1 ± 0.03	1.2 ± 0.03
TU	1.1 ± 0.02	1.1 ± 0.02	1.1 ± 0.02	1.1 ± 0.02
RE	2.3 ± 0.1	2.7 ± 0.1	5.2 ± 0.3	5.9 ± 0.5
PW	1.3 ± 0.1	1.3 ± 0.1	2.0 ± 0.3	2.1 ± 0.1
SN	1.6 ± 0.1	2.0 ± 0.1	2.5 ± 0.3	2.5 ± 0.3
HT	1.2 ± 0.03	1.1 ± 0.02	1.1 ± 0.02	1.1 ± 0.2
SM	2.2 ± 0.1	2.2 ± 0.1	2.9 ± 0.1	2.7 ± 0.1
ST	8.8 ± 1.1	8.6 ± 0.5	8.9 ± 0.6	8.5 ± 1.4
LO	1.5 ± 0.05	1.5 ± 0.1	1.5 ± 0.1	1.4 ± 0.1

[a] Control N = 12 and lead-treated N = 12 per age group, except control N = 8 and lead-treated N = 8 at 4 months.

[b] Significant difference between lead-treated and control groups (t-test, $p < 0.05$).

Table 7-6. SIGNIFICANT EFFECTS OF POSTNATAL LEAD ON PATTERNING OF BEHAVIOR IN MALES[a]

Age	4 Weeks	6 Weeks	4 Months	5 Months
Main diagonal		Decreased $p < 0.01$		
Grooming	Decreased $p < 0.05$			Decreased $p < 0.05$
Exploratory		Decreased $p < 0.01$	Decreased $p < 0.02$	
Attention		Decreased $p < 0.05$		
Grooming linked with exploratory				
Grooming linked with attention				
Exploratory linked with attention		Decreased $p < 0.01$		

[a] Significant differences using the Wilcoxon signed rank test. Occurrences of linked behaviors within 5-second intervals in lead-treated groups are indicated as "decreased" or "increased" in comparison to controls.

Patterning of behavior in lead-exposed females at all ages tested was significantly different from that in corresponding controls (Table 7-7). As in lead-exposed males, more patterning differences in pairing of behaviors within 5-second intervals were observed at 6 weeks of age than at any other age tested. Also as in lead-exposed males, there were significantly fewer occurrences of paired exploratory behaviors and paired exploratory and attention behaviors in 6-week-old lead-exposed females than in controls. However, unlike patterning differences between lead-exposed and control males, pairing of different behaviors in lead-exposed females did not always decrease with respect to controls. More frequent occurrences of different behaviors linking within 5-second intervals were apparent in lead-exposed females at 6 weeks and at 4 and 5 months of age.

Table 7-7. SIGNIFICANT EFFECTS OF POSTNATAL LEAD ON PATTERNING OF BEHAVIOR IN FEMALES[a]

Age	4 Weeks	6 Weeks	4 Months	5 Months
Main diagonal				
Grooming				Increased $p < 0.05$
Exploratory		Decreased $p < 0.01$		Decreased $p < 0.05$
Attention	Decreased $p < 0.05$		Increased $p < 0.05$	
Grooming linked with exploratory				
Grooming linked with attention		Increased $p < 0.01$		
Exploratory linked with attention	Decreased $p < 0.02$	Decreased $p < 0.01$	Increased $p < 0.05$	

[a] Significant differences using the Wilcoxon signed rank test. Occurrences of linked behaviors within 5-second intervals in lead-treated groups are indicated as "decreased" or "increased" in comparison to controls.

Behavior After Amphetamine

There was very little difference in the behavior of controls and the behavior of lead-exposed rats after both had received amphetamine at 6 weeks of age. With respect to frequency (Table 7-8) and duration (Table 7-9) of most behaviors, there were no significant differences after amphetamine between control and lead-exposed rats. This result was similar to that observed prior to administration of drug. Whereas there were many significant differences in patterning of behavior between control and lead-exposed male groups prior to drug (Table 7-6), after amphetamine there appeared to be none. The frequencies with which behaviors were paired in the control optimal sequence and the frequencies with which different behaviors within and between clusters were paired were not significantly different between control and lead-exposed males after both had received amphetamine. However, unlike the males, there were significant differences between control and lead-exposed females in their behavioral patterning both before (Table 7-7) and after amphetamine. The differences after amphetamine consisted of significantly more occurrences, using the Wilcoxon signed rank test, of linked grooming and exploratory behaviors ($p < 0.05$) and of linked exploratory and attention behaviors ($p < 0.05$).

Table 7-8. TOTAL OCCURRENCES OF BEHAVIOR ACTS IN 6-WEEK-OLD RATS AFTER AMPHETAMINE (0.5 mg/kg) AVERAGE ± STANDARD ERROR

	Males		Females	
Behaviors	Control	Lead-treated	Control	Lead-treated
LD	0	0	0	0
SC	3 ± 2	2 ± 1	3 ± 1	2 ± 1
GR	73 ± 12	41 ± 11[a]	43 ± 12	31 ± 10
SI	140 ± 16	108 ± 26	97 ± 17	74 ± 16
WF	60 ± 6	63 ± 15	50 ± 7	41 ± 6
BO	15 ± 3	10 ± 2	12 ± 3	9 ± 1
WA	56 ± 9	42 ± 8	66 ± 5	63 ± 5
TU	38 ± 6	40 ± 8	44 ± 5	56 ± 4
RE	93 ± 11	87 ± 22	108 ± 13	119 ± 20
PW	11 ± 1	13 ± 3	13 ± 2	9 ± 2
SN	28 ± 6	70 ± 24	37 ± 10	52 ± 11
HT	108 ± 8	97 ± 8	110 ± 9	98 ± 4
SM	212 ± 26	220 ± 22	270 ± 16	256 ± 14
ST	610 ± 14	663 ± 27	629 ± 23	645 ± 21
LO	146 ± 20	127 ± 28	132 ± 11	168 ± 14[a]

[a] Significant difference between control and lead-treated groups after amphetamine (t-test, $p < 0.05$).

Table 7-9. DURATIONS OF BEHAVIOR ACTS IN 6-WEEK-OLD RATS AFTER AMPHETAMINE (0.5 mg/kg) AVERAGE DURATION IN SECONDS ± STANDARD ERROR

	Males		Females	
Behaviors	Control	Lead-treated	Control	Lead-treated
LD	0	0	0	0
SC	1.0 ± 0.3	1.5 ± 0.8	1.2 ± 0.4	1.5 ± 0.7
GR	4.4 ± 0.5	3.9 ± 0.5	3.5 ± 0.5	3.2 ± 0.4
SI	13.8 ± 1.5	12.7 ± 1.3	10.5 ± 1.5	6.6 ± 0.6[a]
WF	3.8 ± 0.3	4.5 ± 0.4	4.2 ± 0.4	3.6 ± 0.2
BO	1.0 ± 0.03	1.0 ± 0	1.0 ± 0	1.0 ± 0.01
WA	1.3 ± 0.05	1.2 ± 0.04	1.2 ± 0.04	1.2 ± 0.02
TU	1.1 ± 0.02	1.1 ± 0.03	1.1 ± 0.01	1.1 ± 0.02
RE	2.3 ± 0.2	2.3 ± 0.1	2.5 ± 0.2	2.2 ± 0.1
PW	1.1 ± 0.05	1.2 ± 0.1	1.2 ± 0.05	1.2 ± 0.1
SN	1.3 ± 0.1	2.2 ± 0.2[a]	1.4 ± 0.1	1.7 ± 0.1
HT	1.1 ± 0.02	1.1 ± 0.01	1.2 ± 0.03	1.1 ± 0.02
SM	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
ST	7.8 ± 1.1	11.5 ± 2.3	7.2 ± 0.8	7.2 ± 0.7
LO	1.6 ± 0.1	1.7 ± 0.3	1.5 ± 0.1	1.6 ± 0.1

[a] Significant difference between control and lead-treated groups after amphetamine (t-test, $p < 0.05$).

Discussion

This study was carried out to measure the frequency, duration, and patterning of behaviors in lead-exposed and control rats at various ages and after administration of amphetamine. Since the only major difference between control and lead-exposed rats was in the patterning of their behaviors, routine methods for monitoring locomotor activity would not have been useful in defining this difference. The insignificant differences in frequency and duration of most behaviors in lead-exposed rats in this study were definitely not consistent with frequency and duration changes observed in rats found hyperactive in a residential maze. Rats that were hyperactive in a residential maze (Norton *et al.*, 1976) demonstrated significant increases in frequency of locomotor behaviors, such as bobbing, walking, turning, rearing, pawing, and sniffing. Concomitantly, frequencies of grooming behaviors—lying down, scratching, grooming, sitting, and washing face—decreased, as did the frequencies of attention behaviors—standing, head turning, smelling, and looking. Duration of most behaviors, except that of walking, was shortened in hyperactivity. Perhaps when the frequency and duration changes characteristic of hyperactivity occur, methods measuring activity would be sufficient to consistently detect differences. But in the absence of such changes and when differences exist in behavioral patterning only, results from various activity methods may vary because the methods monitor different behaviors with varying sensitivities for a behavior's frequency and duration.

There is the possibility that if lead-exposed rats in this study had been filmed during the nocturnal period of their light-dark cycle, frequency and duration of their behaviors may have changed in accordance with hyperactivity. This possibility is lessened, however, by the observation that rats with globus pallidus lesions are hyperactive only at night in a residential maze (Norton, 1976); yet when they are filmed during the day, the frequency and duration of their behavior acts are still indicative of hyperactivity. This observation suggests that regardless of when rats are found hyperactive with a maze activity method, basic components of hyperactivity, which can be quantified by time-lapse photographic analysis of diurnal exploratory behavior, may exist throughout the animals' light-dark cycles.

Behavior pattern analysis indicated that the number of intervals when certain different behaviors occurred within 5 seconds of each other in lead-exposed animals was often significantly different from that in corresponding controls. This difference was maximum in the 6-week-old animals. In lead-exposed males at that age, there were fewer 5-second intervals when exploratory behaviors were linked with other exploratory behaviors, when attention behaviors were linked with other attention behaviors, and when attention behaviors were linked with exploratory behaviors. Furthermore, fewer occurrences of behavior pairs comprising the control optimal sequence were observed, perhaps indicating that lead-exposed males had established a different optimal sequence of behaviors. However, further pattern analyses are necessary to test this possibility. There were fewer patterning differences between control and lead-exposed males at the other ages. This peaking of altered behavior pattern at 6 weeks of age may perhaps be comparable to hyperactivity that peaked in young rats postnatally exposed to carbon monoxide (Culver and Norton, 1976) and in children (Weiss *et al.*, 1971); then, in both instances, the hyperactivity diminished with age.

Certain patterning differences between lead-exposed females and their corresponding controls were dissimilar to those between control and lead-exposed males. More frequent occurrences of certain behavior pairs were observed in 6 week and older lead-exposed females, but at no age was this increased frequency of behavior pairing observed in lead-exposed males. These results indicate the necessity of observing lead's effect on behavior in both sexes.

Behavior pattern differences between 6-week-old control and lead-exposed male rats disappeared after administration of amphetamine. After 0.5 mg/kg amphetamine, there were practically no differences in frequency, duration, or patterning of behaviors between control and lead-exposed males. Consequently, the "paradoxical" effect of amphetamine, consisting of decreasing rather than increasing activity in lead-exposed mice (Silbergeld and Goldberg, 1974), was not apparent in lead-exposed rats. There were also no significant differences in frequency and duration of most behaviors and fewer differences in patterning between control and lead-exposed females after amphetamine. However, some patterning differences between lead-exposed and control females were still observed after amphetamine. The higher frequencies of linked grooming and exploratory behaviors and of linked exploratory and attention behaviors suggested that the qualitative effect of amphetamine in lead-exposed females, with respect to their controls, was not the same as that in lead-exposed males.

This study demonstrates a situation in which quantifying changes in behavioral patterning is paramount to simply quantifying changes in activity levels. It is possible that other CNS toxicants besides lead predominantly affect behavioral patterning, and, if so, the analysis of patterning with time-lapse photography becomes more than just a sensitive technique; it becomes a necessity.

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8. BEHAVIORAL ASSESSMENT IN SENSORY TOXICOLOGY

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Sensory impairment, such as numbness or blurred vision, is a consequence of exposure to any of a large variety of substances (see Grant, 1974, or Foulds, 1974). In the toxicology of sensory impairment, the behavioral scientist may be able to offer more precise definition of impairment, including improved sensitivity and reliability of tests, with the ultimate hope of providing better differential diagnosis, early detection of intoxication, and more intelligent environmental health standards. This chapter reviews early indications of this potential and examines behavioral principles likely to aid the precise definition of sensory impairment caused by toxins.

Sensory function is a particularly apt behavioral process for toxicology because of well developed psychometric procedures relating the behavioral response to physical qualities of the stimulus, while segregating nonsensory variables. Some of the earliest yet most complete behavioral studies involved auditory and visual impairment (see reviews by Stebbins and Coombs, 1975; and by Hanson, 1975). Orderly psychometric curves demonstrated a selective sensory impairment related to a lesion caused by the drugs; they illustrate the utility of probing the controlling parameters and the use of psychophysics to specify sensory impairment after initial stages of drug screening. It is not necessary to review this body of work again here, but a reading of these studies and of the book *Animal Psychophysics* (Stebbins, 1970), should precede behavioral studies involving sensory toxicology.

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Reviewing behavioral studies of sensory impairment reveals some principles of general utility in the diverse endeavors known as Behavioral Toxicology. The paramount principle is the importance of determining specific mechanisms of action, both at the behavioral and biological levels of analysis. Unfortunately, too many studies in behavioral toxicology are purely descriptive, with neither an attack on underlying mechanisms nor a clear extrapolation to human health questions. Our sibling discipline of sensory psychopharmacology evokes criticism on similar grounds (Robinson and Sabat, 1975). Some behavioral toxicologists appear to be neglecting mechanisms while seeking a threshold dose in hope of observing an effect at a dose lower than previously reported. The study of basic mechanisms is not incompatible with the search for threshold doses. The pursuit of the mechanisms (i.e., the controlling variables) will ultimately improve a behavioral test's sensitivity to low doses. This is my message.

Why examine specific behavioral mechanisms of action? One reason is to improve the basis for pursuing underlying neural mechanisms and for extrapolating to humans. Since humans cannot be subjects for toxicity experiments, the applicability of animal data to humans must be assessed upon some basis other than direct species comparison. A similar limitation has not prevented great progress in the neurophysiology of vision, particularly since the pioneering work of Hubel and Wiesel (1962). Behavioral tests of sensory function identified behavioral mechanisms common to humans and laboratory animals. Then brain recordings and manipulations, with experimental animals, suggested human neural mechanisms as well as new paths of human behavioral research. Behavioral toxicology should provide a similar liaison between basic laboratory experiments and clinical studies with humans, with cross-reference between behavioral and biological analyses.

Using this approach in our studies of methylmercury, we identified similar visual functions in humans and normal monkeys, then determined the specific visual impairment in monkeys that suggest early warning signs of intoxication in humans (Evans, 1975b). The pattern of mercury distribution in the brain and the rate of elimination also are being explored in search of biologic mechanisms that would account for the primate's greater sensitivity to methylmercury as well as its greater susceptibility to sensory impairment compared to subprimate species (Evans *et al.*, 1977; Evans and Laties, in preparation).

Knowledge of basic behavioral mechanisms also is useful in dealing with special kinds of problems encountered in assessing toxins, problems seldom encountered in assessing psychopharmacologic agents. A problem in examining both biological and behavioral measures is to define a "toxic effect," i.e., to discriminate a true impairment from a "change." Several recent papers, that I found to be interesting and better than average in other respects, illustrate this point.

The Iowa group used a variation of Stebbins' auditory procedure and found that the pesticide, parathion, increased the variability of the monkey's auditory threshold (Reischl *et al.*, 1975). Should this be considered a toxic effect in the sense of greatest concern in making health decisions? A finding of this sort should be examined with one of the procedures discussed below that can help separate specific sensory effects from nonsensory effects. Reischl's monkey was to press a lever when he heard a tone and to do nothing in the absence of a tone. There is a hazard associated with using two markedly different kinds of response to indicate the two choices in a discrimination. Behavioral pharmacologists have found that many drugs can change the rate of probability of some behaviors' occurrence, depending upon the details of the test situation (see reviews by Sanger and Blackman, 1976; Kelleher and Morse, 1968). Response-rate changes induced by toxins need not be related to sensory impairment. Suppose the toxin changed the probability of occurrence of all behaviors. This would change the proportion of trials in which the monkey does nothing, i.e., indicates "I don't hear anything," without necessarily changing auditory sensitivity. Forcing the monkey actively to select one of two levers to indicate the presence or absence of the stimulus would have permitted the calculation of the percentage of correct discriminations independently of changes in response probability. Then, no default choice need be inferred from a withheld response. This approach has been illustrated with anticholinergic drugs (Evans, 1975a).

The findings of Reischl *et al.* (1975) suggest a characteristic of threshold or near-threshold doses: the problem of weak and variable results. An apparently toxic effect may wax and wane before becoming clear (e.g., Berlin *et al.*, 1975). If the basic mechanism of action were pursued first, even if higher doses were required to do so, we would have a basis for deciding whether the weak effect, seen after the administration of a low dose, represents a logical extrapolation of the clear effects determined previously.

As the discussion above suggests, attempts to determine whether an effect is a true "toxic" effect lead to a related problem: determining specificity of the result. Behavioral measures such as "spontaneous" locomotor activity or multiple (FI/FR) response rates make excellent screening devices because they may be influenced by any toxin that produces "sickness." Used in this way, changes in response rates reveal little about controlling mechanisms. The skills and interests of the behavioral scientist are more fully engaged by questions such as "Can a toxin's effect be said to involve sensory, motor, or some intellectual mechanism?"

Categories of behavioral disruption (e.g., impairment of discriminative learning), like disease symptoms, are not specific to a single causal factor but may occur as the result of a variety of causes. Young rats treated with lead showed an intriguing impairment in the learning of a brightness discrimination although they ultimately performed at a level characteristic of the control animals (Brown, 1975). As with earlier studies in this area (Carson *et al.*, 1974), perhaps the next question should be "Could this represent a sensory deficit, with the brightness discrimination learned more slowly because the animals didn't see very well?" There seems to be some evidence that lead may, some visual functions (Bushnell *et al.*, 1977), as does another heavy metal, methylmercury (Evans *et al.*, 1975). Since Brown's rats were required to run through a T-maze, the learning impairment may also reflect changes in motivation or motor coordination. In a similar experimental situation, impaired learning of a brightness discrimination by rats exposed to tellurium was attributed to motivational rather than sensory impairment, since impairment was also present in a one-way shock avoidance that involved no discriminative stimuli (Dru *et al.*, 1972).

Acquisition of maze-running is influenced by many variables, such as baseline level of motor activity, which may be changed by lead exposure (Silbergeld and Goldberg, 1974) without reflecting either learning or sensory capabilities. The influence of activity level upon the speed of rats' maze-learning has been more fully examined in the area of genetic differences (Ray and Barrett, 1975). Thus, Brown's report (1975) leaves us far from a conclusion that early exposure to lead retards discriminative ability, although many people would agree that whatever the ultimate nature of the effect, it is likely to be classified as toxic.

We must pursue these basic mechanisms to insure the greatest success beyond the initial screening stage. Psychologists long have been struggling with questions such as learning versus memory versus performance. Behavioral toxicology needs input from those who are conversant with this literature. Several contributors to this volume have indicated the wisdom of pausing to check the literature instead of "reinventing the wheel." The following pages review some principles of behavior that can improve our behavioral assay by increasing sensitivity to low doses and by permitting greater specification of response classes. Only then can we say with confidence exactly what aspect of behavior is changing and whether the change is good, bad, or indifferent.

The Signal Detection Approach

Signal detection theory arose from engineering and illustrates many ways in which we can be confused when estimating a change in sensory capability (e.g., Green and Swets, 1966; Swets, 1973; Wright and Nevin, 1974). Possible sensory changes can be confounded with changes in nonsensory factors, such as response position bias, motivation, guessing, or in the motor coordination in the execution of the required response. These changes can alter the reporting of a sensory stimulus and thus can be confounded with the true sensory impairment.

One of the main advances of the signal detection approach was to yield a pure measure of sensory capability (d'), with the nonsensory factors lumped together in a second measure, termed "bias." However, even when the stimulus is held constant, this supposedly pure, quantal measure of sensitivity varies with variations in the reinforcement contingencies employed in the test (Nevin, 1975), as does practically every other behavioral measure. Even if the signal detection paradigm is not perfect, its emphasis upon segregating the influence of bias provides a basis for improving some of the previous approaches to sensory toxicology.

The only application of signal detection methods thus far in behavioral toxicology is the suggested use of olfactory detection to improve the monitoring of air pollution (Berglund and Berglund, 1974). More experiments employing traditional signal detection are found in pharmacology. In the most thorough animal experiment to date, LSD was found to alter response bias of rats without changing the auditory sensitivity (Dykstra and Appel, 1974). These drug-induced changes resembled those produced by manipulations of reinforcement probabilities. This study illustrates the need for caution in applying signal detection procedures, developed by using trained human observers, to studies with animals exposed to drugs and toxins. The common tendency of animals to withhold responding on a proportion of the trials and for drugs and toxins further to reduce the response probability can result in a paucity of data with which the originators of human signal detection methods were not forced to contend.

Signal detection methods may help to separate changes in cutaneous sensitivity from changes in verbal reporting following analgesia (Chapman *et al.*, 1973; Clark and Yang, 1974). However, the meaning and validity of these examples have been questioned in letters from numerous authors (see *Science* 189, 65-68, 1975). Pastore and Scheirer (1974) have reviewed other limitations in signal detection application.

The signal detection analysis, as most commonly employed, has additional limitations. Although the paradigm yields quantal results, one must be cautious not to violate the assumptions on which the statistical calculations are based. One hopes that the opportunity to obtain a quantal index of sensitivity, by plugging numbers into one of the formulae available, doesn't promote unwarranted complacency.

The signal detection approach most commonly employs an extremely simple behavioral task, with a stimulus either "on" or "off," and two responses equivalent to "yes I see it," "no I don't see it." Many questions in behavioral toxicology require manipulation of other stimulus parameters and of reinforcement contingencies in order to probe fully the variables of interest. The following sections describe these alternatives. In summary, signal detection theory is stimulating a helpful reappraisal of experimental design. The classic procedure offers an alternative approach in assessing sensory impairment; its utility in behavioral toxicology is still uncertain.

Complex Discriminative Behavior

A discriminative technique of long standing in primate research, the WGTA (Wisconsin General Test Apparatus) was employed in assessing chronic exposure of squirrel monkeys to methymercury (Berlin *et al.*, 1973; 1975). The monkey was required to discriminate between two stimuli, which were three-dimensional objects, by pulling a chain attached to the stimulus. This procedure, like the maze discussed above, may reflect changes in both sensory and motor systems, requiring an astute experimenter to distinguish between changes in response speed, visual-motor coordination, accuracy of discrimination, etc.

It will be useful in further discussions to consider the WGTA task in two components: (1) the visual stimuli and (2) the required response. Berlin's discrimination could be considered relatively easy, since the two stimuli differed in each of three visual dimensions: color, form, and size. The choice response could be considered relatively complex and difficult, since the monkey first had to select the end of one of two chains attached to the stimuli, then use the chain to pull the stimulus object toward him and thus retrieve a piece of food. The point I wish to make here is that the accuracy of the visual discrimination was no more revealing of the early effects of methylmercury than were the performance measures embodied in the chain-pulling sequence.

More subtle types of visual impairment were identified by subsequent experiments as the earliest signs of methylmercury intoxication in primates (Evans *et al.* 1975; Berlin *et al.*, 1975). The critical results were obtained by manipulating the strength of the discriminative stimuli. Since motor coordination had been shown not to be among the early toxic signs in primates (Evans *et al.*, 1975), a simple operant response (button-pressing) was employed in order to minimize the motor complications in evaluating visual impairment. Exposure to methylmercury, in doses much like those used previously, increased the critical fusion intensity; i.e., the luminance required for a flickering light to be discriminated from a steady light increased as exposure progressed (Berlin *et al.*, 1975). The frequency of flicker was held constant while luminance was varied. This reaction was interpreted as a loss of scotopic (night vision) sensitivity to light.

The critical flicker frequency test (CFF) is complementary to the critical fusion intensity test described above. In determining the CFF, luminance is held constant while frequency is manipulated until the flicker disappears. Although toxins have not been widely studied with CFF, the majority of psychoactive drugs tested in humans cause a decrease in the critical frequency (Smith and Misiak, 1976).

Another aspect of visual impairment occurring during the early phase of methylmercury intoxication was assessed with a visual discrimination test devised in our Rochester laboratories. The procedure was planned to facilitate the distinction between sensory and nonsensory effects illustrated in discussing the signal detection approach. We tried to make the discrimination procedure more informative than the "yes-no" detection paradigm by requiring the animals to choose from among three discriminative stimuli, instead of between the customary two choices. This change expanded the range of scores between perfect (100 percent) and chance (33 percent correct with three choices but 50 percent correct with two choices). Similarly, the three response choices provide an expanded baseline for identifying nonsensory factors such as response position bias (Evans, 1975a). We achieved a quantal index of strength of the controlling stimuli by varying the luminance of the stimuli in steps instead of in an all or none fashion, as was customary in previous drug studies. The resultant psychometric functions can be used to estimate the strength of the stimulus control of behavior and to relate this to a quantal property of the stimulus, (Evans, 1975a,b).

Strength of Stimulus Control as a Predisposing Factor

Experiments with operant behavior have contributed a substantial understanding of how environmental stimuli can influence (i.e., "control") behavior. Experiments in this tradition have indicated that effects of drugs and toxins vary with the extent to which behavior is controlled by stimuli and reinforcing contingencies (Laties, 1975; Evans and Weiss, 1978). The following examples illustrate how strength of control by visual stimuli can be a predisposing factor in behavioral toxicology. I believe the principle applies to other sensory modalities, but more evidence is required.

Visual discrimination under the control of weak stimuli (low luminance) was more vulnerable to disruption by drugs and toxins than was similar discriminative behavior controlled by strong stimuli. Impairment of discrimination with weak stimuli (low luminance) was the first sign to emerge during chronic methylmercury exposure (Evans *et al.,* 1975; Evans, 1975b), which was in agreement with findings reported at the same time by Berlin *et al.* (1975). Generality for the conclusion was obtained in acute studies with anticholinergic drugs as reference componds (Evans, 1975a). Discriminative accuracy with weak controlling stimuli was impaired by a dose of scopolamine one-fourth that required to impair accuracy slightly with a strong stimuli. These findings may be related to the fact that visual deficits caused by brain lesions vary, depending upon the eye's adaptation to ambient illumination (Glickstein *et al.,* 1970; Weiskrantz and Cowey, 1967).

Another type of two-choice visual discrimination procedure was used to assess the effects of parathion (Reiter *et al.,* 1975). All effective doses abolished responding and markedly reduced blood cholinesterases within 5 hours; complete recovery usually occurred within several days. Several factors suggest that visual processes probably were not involved in these results. First, parathion reduced response probability without markedly changing accuracy of discrimination. Several variants of the discrimination problem, each with different stimulus patterns, revealed no differential effect relating to stimulus complexity or task difficulty. These predisposing factors will be discussed below. Second, the abolition of responding was accompanied by marked, nonvisual toxic signs. Third, *both* reference drugs, scopolamine and methylscopolamine, produced an abrupt cessation of responding similar to the effect of parathion. Involvement of the eye or the brain would be indicated by a marked difference in the effectiveness of the two drugs (see Evans, 1975a).

Strengthening the stimulus control in an operant task with pigeons could improve the performance that had been degraded by methylmercury (Laties, 1975). Manipulating stimulus control showed that the toxic effect involved stimulus, rather than motor, mechanisms, since the pigeons' response patterns returned to baseline following an increase in the strength of the controlling stimulus.

The utility of manipulating an aspect of stimulus strength is also illustrated in a preliminary report of a selective decrement of visual acuity. Lead-exposed monkeys, that had been considered "normal" after showing no deficits under high illumination, showed a deficit when later tested under low illumination (Bushnell *et al.*, 1977). This effect of lead resembles that reported for methylmercury (Evans *et al.*, 1975; Evans, 1975b), a surprising finding since neither behavioral nor biological mechanisms of inorganic lead intoxication has been reported to resemble those of methylmercury.

The above evidence indicates two benefits in manipulating strength of stimuli. First, it increases the sensitivity of the test to low doses and to early, preclinical effects in chronic exposure, such as those with methylmercury (Evans, 1975b). Second, manipulations of stimulus strength facilitate conclusions about specific sensory mechanisms, with nonsensory factors considered separately. For example, demonstrating a relationship between a toxic effect and a specific stimulus parameter, e.g., luminance, argues against an influence of memory, motivation, or motor coordination, since these factors can be controlled during manipulation of the stimulus parameter (e.g., Evans, 1975a; Evans *et al.*, 1975).

Behavioral toxicology in the Soviet Union may contain evidence supportive of the above conclusions. Medved *et al.* (1964) review evidence that conditioned responses elicited by weak stimuli are more easily disrupted by a pesticide than are responses to stronger stimuli. Trakhtenberg (1974) summarizes studies characterizing early effects of mercury vapor, including a greater increase in response time to "a weak stimulus" (a white light) than to a "strong stimulus" (buzzer). Later stages of intoxication were characterized by a "release of differentiation" (differential responses were no longer controlled by the discriminative stimuli), followed by anorexia, lethargy, etc. Soviet scientists also have employed olfactory and visual sensitivity in assessing sulfur dioxide and related gases (see Xintaras and Johnson, 1976, for a summary). Appraisal of the Soviet literature is difficult for the average Western scientist because of the unfamiliar vocubulary, which represents an insurmountable barrier if sufficient details of methods and results are not provided. One is uncertain as to whether "weakening of internal active inhibition" or "cortical inhibition spreading to the sub-cortical region" (Trakhtenberg, 1974) refers to any of the mechanisms discussed here. Trakhtenberg cites some startling positive behavioral findings with extremely low concentrations of mercury vapor, but these reports apparently are not published in the archival scientific literature.

The Complexity of the Stimuli

Thus far we have focused on the strength or intensity of stimuli as a predisposing factor in determining sensitivity to toxins. It may be profitable to consider complexity of the stimuli as an additional predisposing factor. Together, strength and complexity of the stimuli seems to account for much of what might be referred to as the "difficulty" of sensory tasks. Difficulty could be related to the rate of reinforcement under control conditions, i.e., the percent of correct choices. Our work with methylmercury and visual discrimination is accumulating evidence of this relationship. When stimulus strength (luminance) is held constant, discrimination of relatively complex stimuli (3 stimuli of equal area but different shape) seems more vulnerable to disruption than is the discrimination of less complex stimuli (selection of the brightest of 3 stimuli without regard to form). Our monkeys are tested equally often on both discrimination tasks. Psychophysical data from normal monkeys and humans, as well as from monkeys exposed to methylmercury, suggest an interesting interaction between strength and complexity of the stimuli.

Rats showed impaired visual pattern discrimination after exposure to tellurium, although a simple brightness discrimination was not impaired (Dru *et al.*, 1972). As before, more evidence exists in drug studies of sensory impairment reviewed by Laties (1975). Additional studies have shown that alcohol selectively impaired detection of both auditory (Moskowitz and DePry, 1968) and visual (Moskowitz and Sharma, 1973) stimuli only when the task complexity was increased. Replication of this work is described by Xintaras and Johnson (1976). Additional manipulations of task difficulty in discrimination research have been described by Ettlinger and Rashbass (1976).

Behavioral toxicologists should consider these and other variables influencing task difficulty to be of basic importance as well as of practical utility. Task difficulty may be a determinant of the behavioral effects of toxins as it has long been recognized as a determinant of impairment following brain lesions: performance of a very easy task is refractory to lesions that are sufficient to eliminate performance of very difficult tasks (Lashley, 1929; Chow, 1967). Most psychologists recognize Lashley's efforts to identify behavioral impairment associated with removal of large areas of the rat's brain; he found that as much as one-half of the brain could be removed without altering the rat's ability to solve some mazes, illustrating that performance decrement is a joint function of task difficulty and the amount of brain damage (Lashley, 1929), a 50-year-old fundamental principle that should be of utility to the fledging discipline of Behavioral Toxicology.

Conclusions

Sensory function has a good potential for behavioral studies in toxicology, whether the prime interest is in developing a very sensitive assay or in defining specific sensory mechanisms. A review of recent reports with methylmercury, lead, and parathion reveals that, empirically, sensory function seems to be sensitive to a wide variety of toxic agents. Compared with other psychological phenomena, changes in sensory performance can be more clearly interpreted as "toxic" and seem to be relevant to questions of human health and safety.

Manipulation of the behavioral parameters should be considered of importance equal to the manipulation of the dose parameters. The parametric curve, such as the psychophysical function, should have a standard place along with the dose-effect curve in behavioral toxicology.

The concept exemplified by the signal detection approach merit inclusion in all assessments of sensory function instead of the classic detection procedure, even if the experiment requires one of the discriminative tasks described herein. All of the procedures employ a discrete-trial, rather than free-operant schedule, to obviate the need to infer sensory function from changes in response rates.

Our legacies in neuropsychology and in behavioral pharmacology offer useful discriminative procedures and suggest that the task difficulty, resulting from strength and complexity of the stimuli, will be an important factor in assessing sensory impairment.

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9. ELECTROPHYSIOLOGICAL TECHNIQUES IN TOXICOLOGY

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An Overview of "Electrotoxicology"

Most poisons are neuropoisons. Poisons which act upon the nervous system are particularly effective in producing toxicity because the nervous system is more sensitive to disruption of function than are other physiological systems. At the same time, the organism is dependent for survival on an intact and functioning nervous system. Thus, hypoxia will result in death of neurons before death of other cells in the body, which, in turn, may lead to death of the organism if the destroyed neurons normally regulate vital functions, such as respiration. This combined sensitivity and vital importance of the nervous system has been known for a long time. It has become evident, particularly in the last decade, that low-level exposure to a toxicant may produce deleterious neural effects which are discovered only upon the utilization of sensitive, sophisticated testing techniques. The concern today is not so much with neurotoxicants which produce death immediately, but with the fear that environmental toxicants may reduce functioning of the nervous system in a less obvious, but still important, way, so that intelligence, memory, or other higher central neural functions are affected. With this realization has come the birth of behavioral toxicology.

Behavioral toxicologists are frequencly under pressure from other toxicologists, who often either don't understand or don't trust behavioral tests, to relate changes in behavior to changes in other physiological or biochemical endpoints. Therefore, it is to the advantage of the behavioral toxicologist to measure as many parameters of nervous system activity as is feasible and to relate these to changes in other physiological systems. Among these other parameters of nervous system functioning which may be utilized to detect and understand neurotoxic effects are electrophysiological measurements. This chapter is a brief attempt to point out some of the advantages and limitations of electrophysiological recording techniques.

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Electrophysiological techniques may be relatively more sensitive indicators of toxic action than are other parameters. Of course, to be sure that this is true, other parameters must be measured along with the electrophysiological recordings. Electrophysiology may be a useful tool to determine some aspects of the mechanisms of action of toxicants. It is possible to implant electrodes in various cortical and subcortical brain structures and then to utilize evoked potential techniques to test the integrity of specific brain systems or parts of brain systems. The use of microelectrode techniques to determine the effects of toxicants at the cellular level has proven to be an immensely successful procedure for elucidating specific types of membrane or synaptic effects of toxicants. For example, research has demonstrated that DDT is an axon labilizer, i.e., it prolongs the action potential negative afterpotential or causes repetitive firing of action potentials, probably by prolonging the increased sodium conductance which accompanies the spike potential and which normally declines during the falling phase of the action potential (Narahashi, 1971). Also, lead ions have been shown to block transmission in the superior cervical or other sympathetic ganglion and neuromuscular junction, probably by reducing release of acetylcholine from presynaptic terminals (Kostial and Vouk, 1957; Cooper and Manalis, 1974; Silbergeld *et al.*, 1974). However, the electrophysiological technique most useful to a behavioral toxicologist is probably that of using animals with chronically implanted brain electrodes, so that the effects of an agent on spontaneous and evoked brain electrical activity may be recorded in awake unanesthetized animals, preferably in association with a behavioral task. Recently, event-related slow potentials recorded from the cortical surface with nonpolarizable electrodes have been used to investigate the effects of drugs on behavior and electrophysiology (Pirch and Osterholm, 1975). These potentials should prove useful for the study of toxic agents.

Because chronically implanted rats are so useful for neurotoxicological studies, a description of some of the procedures for implantation of brain electrodes which have been developed in the author's laboratory is presented below. Descriptions of additional procedures for electrophysiological studies are also available, e.g., Skinner, 1971.

Preparation of Rats with Chronically Implanted Brain Electrodes for Studies in Toxicology

The use of animals with chronically implanted brain electrodes makes it possible to study the effects of an agent in an unanesthetized animal. This technique has the obvious advantage of eliminating the complicating factor of interaction between toxicant and anesthetic, and it permits comparison of changes in brain electrical activity with changes in spontaneous or experimentally modified behavior. Rats are particularly convenient to study because a chronically implanted rat may be "plugged in" for recording (Figure 9-1) in its home cage and still have the same degree of freedom to move about that it normally does (Figure 9-2). In this way the novelty of the recording situation is minimized.

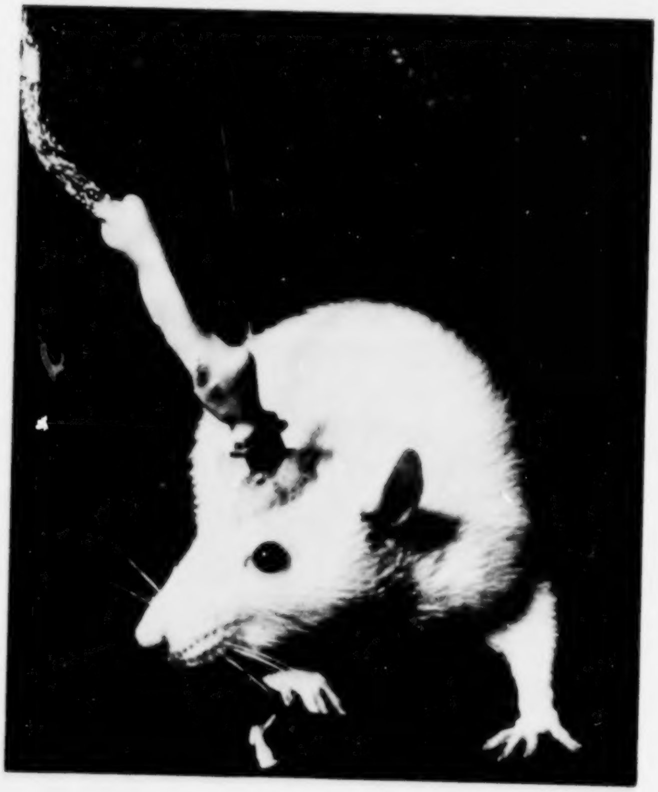


Figure 9-1. Rat with chronically implanted brain electrodes plugged in for recording. The electrodes in the brain are connected to the terminals of a subminiature connector and are held in place by dental acrylic. The recording cable connects the recording equipment to the brain electrodes via a subminiature connector which is the mate to the one on the rat's head. The initial portion of the recording cable is covered with a light weight rubberized material to give some rigidity to the cable.

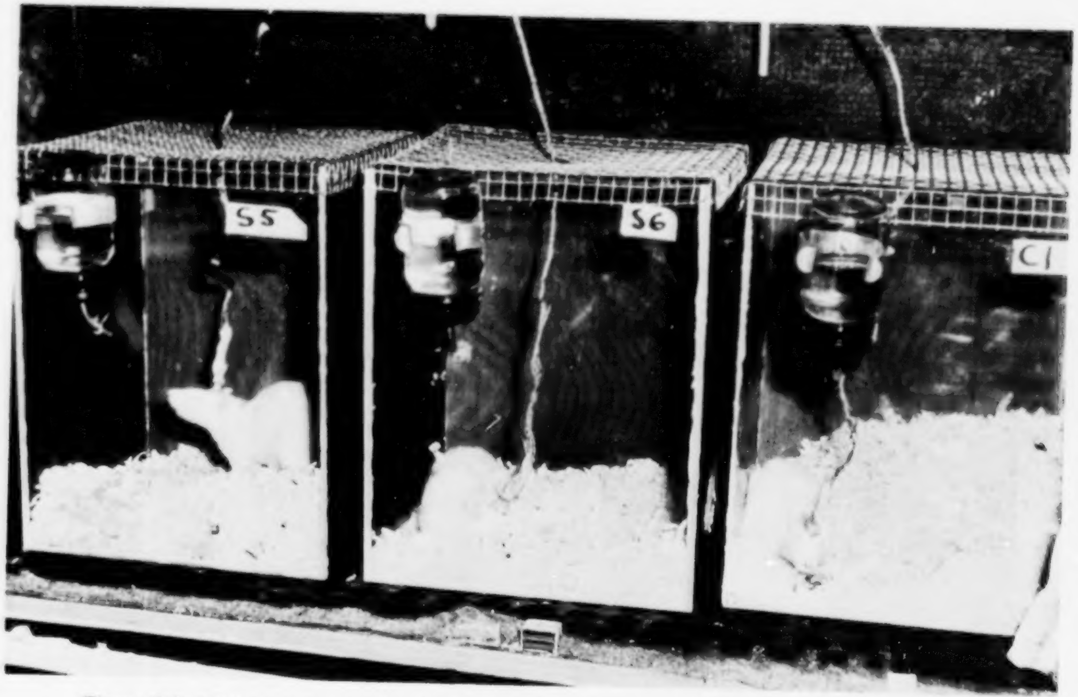


Figure 9-2. Tall cages are used as home cages so that implanted rats cannot bump their connectors against the top of the cage. Rats may be plugged in and recorded in these cages to minimize the novelty of the recording situation.

For chronic implantation, side-by-side electrodes with 0.5-1.5 mm tip separation (Figure 9-3) are usually used. Thirty-two gauge stainless steel wire coated with insulating varnish (Diamel) is stretched slightly to straighten it, and lengths are cut with fingernail clippers, which readily cut wire of this gauge without bending the tips. Two wires are held together with a drop of epoxy adhesive (fast curing, 2-part epoxy) so that one of the tips is longer than the other (Figure 9-3). The wires are dipped in an instantly drying adhesive containing cyanoacrylate (Krazy Glue) to hold the wires below the bead together. Next, they are dipped in Epoxylite (No. 6001-M, electrode insulator, Epoxylite Corporation) and baked (3 hours, 150°C). After repeating the dipping and baking, the long electrode tip is cut off with the clippers to give the desired tip separation, usually 1.0 mm, and the tips of the electrodes are bared with a grinding wheel, using a hand-held hobby drill. The other ends of the electrodes are scraped clean, and each is inserted into a steel tube epoxyed onto a thin piece of bakelite (or other plastic) held by epoxy to a steel bar (Figure 9-3), which fits into the standard electrode carriers associated with any stereotaxic apparatus. Wires from the steel tubing connect to stimulating and recording equipment so that any bipolar electrode may be used for either electrical stimulation or recording during positioning of the electrodes. Electrodes are positioned in various brain structures with the aid of stereotaxic equipment.

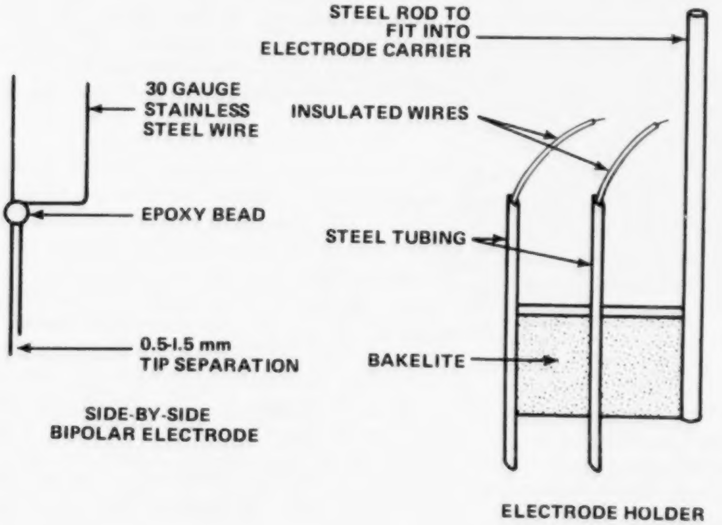

STEEL ROD TO FIT INTO ELECTRODE CARRIER
30 GAUGE STAINLESS STEEL WIRE
INSULATED WIRES
EPOXY BEAD
STEEL TUBING
0.5-1.5 mm TIP SEPARATION
BAKELITE
SIDE-BY-SIDE BIPOLAR ELECTRODE
ELECTRODE HOLDER

Figure 9-3. A bipolar, side-by-side electrode and electrode holder constructed as described in the text. The upper portion of each of the two wires which from the electrode is scraped clean and inserted into the tubing on the bakelite section of the electrode holder. The tubing is connected via insulated wires to the stimulating and recording equipment. The section of bakelite is narrow so that two or more electrodes may be positioned simultaneously over the rat's head.

Evoked potential techniques are utilized to assist in accurate placement of both stimulating and recording electrodes. First, the stimulating electrode is positioned approximately using stereotaxic coordinates, and the bipolar recording electrode is lowered until the evoked potential recorded from each of the recording tips is nearly optimal. Then, the stimulating electrode is adjusted slightly to improve further the elicited response. In other words, the stimulating and recording electrodes are first positioned approximately with stereotaxic coordinates, and the positions of each are refined using evoked potential techniques. By stimulating the olfactory bulb or lateral olfactory tract, it is possible to place bipolar recording electrodes so that the tips straddle the prepyriform pyramidal cell bodies and the evoked potentials recorded from the electrode tips are mirror images of each other, i.e., are 180 degrees out of phase (Woolley and Timiras, 1965). This requires placing the long electrode tip in the prepyriform superficial dendritic layer with an accuracy of a few tenths of a millimeter, a feat which would be impossible using stereotaxic coordinates only. Similarly, accurate placement in the hippocampus may be achieved by stimulating the prepyriform cortex and lowering a bipolar recording electrode into the hippocampus until a phase reversal of the evoked potential between the two recording tips is observed (Woolley and Barron, 1967). For positioning electrodes in the superior colliculus, light flashes may be used to elicit visual evoked potentials during implantation. An electrode is lowered into the superior colliculus until a phase reversal of the elicited potential indicates that the long electrode has penetrated the stratum opticum of the superior colliculus. The reference electrode used for comparing monopolar recordings from each tip of a bipolar electrode is usually formed by a wire wrapped around each of 4 screws positioned at the outer edges of the top of the skull.

After the electrodes are positioned, small screws are threaded into holes drilled into the skull. The electrodes are held in place by flowing dental acrylic around the electrodes and screws. Ends of the electrodes are connected to the pins of a subminiature socket or plug connector (for example, ITT Cannon subminiature connectors MD1-9SL1 or MD1-9PL1), either by soldering or by tightly wrapping the electrodes around the connector pins. During recordings, after recovery from the implantation procedure, another subminiature connector, which is the mate to the connector on the rat's head, is used to plug in the rat and connect the implanted electrodes to amplifying, stimulating, and recording equipment via a recording cable (Figure 9-1). Implanted rats are housed individually in specially tall plastic cages (14" high x 12" x 12") so that the rat does not bump the connector on top if its head against the top of the cage. Side panels of the cages are painted black so that the animals are not distracted by seeing rats in adjacent cages. The front panel is clear so that the experimenter may view the animal from the front as well as the top (Figure 9-2).

In examining the effects of toxicants on brain electrical activity, both spontaneous electrical activity (SEA), i.e., EEG type of activity, and evoked potentials are recorded. For both, recordings are usually bipolar. Monopolar recordings are sometimes carried out in addition to determine if recordings from one of the bipolar electrode tips is altered more than the other. For monopolar recordings, the reference electrode is formed by a wire wrapped around several screws in the skull, as described above. For the findings described below, most recordings were bipolar.

It is well known that changes in behavioral state are correlated with changes in spontaneous and evoked brain electrical activity. Therefore, in evaluating the effects of an agent on brain electrical activity, the behavioral state during the recordings must be noted. It is not unusual for a toxicant to alter the level of arousal. In order to be able to evaluate whether or not the changes in brain electrical activity produced by an agent are related to changes in arousal, it is necessary to carry out control recordings during inattentiveness and arousal so that the patterns characteristic of these states can be recognized and compared with the patterns produced by the toxicant.

Examples of Electrophysiological Studies in Toxicology

Effects of DDT on Brain Spontaneous Electrical Activity (SEA)

The effects of a single dose of DDT, dissolved in corn oil administered *per os* by intubation, on brain SEA and on spontaneous behavior were studied in rats with chronically implanted brain electrodes (Woolley and Barron, 1968; Woolley, 1970). The single dose LD_{50} for DDT was 250 mg/kg, and a dose of 50 mg/kg was the threshold dose for producing grossly observable symptoms. Hyperexcitability, as evidenced by a startle response to sudden sound, such as a hand clap, was usually present at the time of maximal effect of DDT, which was about 8 to 12 hours after administration of 50 mg/kg. In about half of the animals, mild, whole-body tremors were also evident at this dose.

When 50 mg/kg DDT were administered, the frequency of the slow waves in the SEA of the olfactory bulb increased steadily from 4 through 12 hours (Figure 9-4) and then decreased again to reach control levels at 24 hours. These slow waves track respiration (Woolley and Timiras, 1965,), and an increase in respiration is a sensitive early effect of low doses of DDT in the adult rat (Henderson and Woolley, 1969). Frequency of the respiratory waves was 330/min 8 hours after DDT in the example shown in Figure 9-4, compared with 120/min during the control period.

Another significant and sensitive effect of DDT was an increased occurrence and amplitude of the high frequency "arousal" waves in olfactory electrical activity, as shown in Figure 9-5. These fast (55 to 75 cycles/sec) waves are called arousal waves because they occur when the animal is excited, decrease when he is inattentive, and disappear when he is asleep. During the stimulatory stages of DDT poisoning, these waves were consistently present and increased dramatically for hours at a time. This was, in fact, one of the most unique effects of DDT. DDT administration also increased the occurrence and regularity of 7/sec frequency theta waves and increased the amplitude of a fast frequency of about 40/sec superimposed over the theta waves in the hippocampal SEA (Figure 9-4).

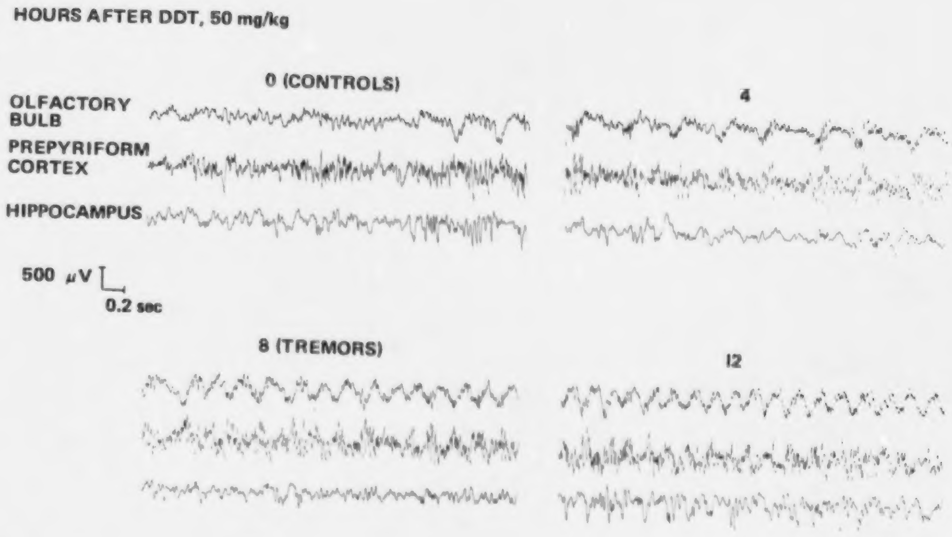


Figure 9-4. Spontaneous electrical activity recorded from the olfactory bulb, prepyriform cortex, and hippocampus in awake, unrestrained rats before and 4, 8, and 12 hours after *per os* administration by intubation of p,p′-DDT dissolved in corn oil.

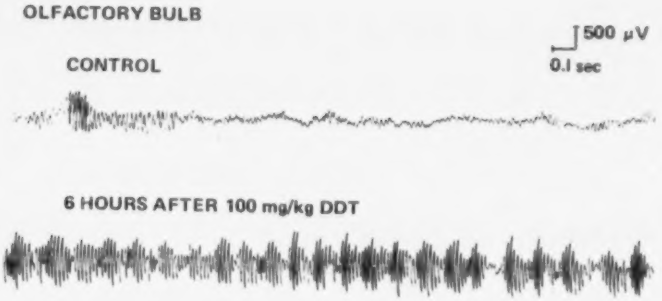


Figure 9-5. High frequency "arousal" waves recorded in the spontaneous electrical activity of the olfactory bulb of the rat before and 6 hours after DDT.

Still other effects of 50 mg/kg DDT on the SEA are shown in Figure 9-6. During the control period, a sudden noise caused the high amplitude slow waves characteristic of an innattentive animal to change suddenly to low amplitude, high frequency waves in the SEA of the frontal cortex, occipital cortex, reticular formation, and vermis of the cerebellum. These are the typical arousal patterns for the SEA of these brain areas. The effects of the relatively low dose of DDT were to cause high frequency "arousal" waves to be continuously present, even in the absence of an arousing stimulus, but these waves had a significantly higher amplitude than in the usual arousal state. In addition, DDT also caused the appearance of "spiking" in the SEA of the cerebellum. High amplitude spikes with about the same frequency as that of the whole-body tremor were pronounced in cerebellar recordings even in the mild tremors produced by 50 mg/kg DDT (Figure 9-6), but the spikes were not usually present in recordings from other brain areas. The changes in cerebellar electrical activity appeared to support earlier suggestions that the cerebellum is a primary target for the action of DDT and that cerebellar dysfunction is basically involved in the symptomatology of DDT poisoning (see review by Hayes, 1959).

Comparison of the effects of a higher dose of DDT (100 mg/kg *per os*) on the SEA of the neocerebellum (ansiform lobule), the cerebellar vermis (lobulus simplex), and the medial and lateral portions of the somesthetic cortex (Figure 9-7), revealed that amplitude of the electrical activity of the neocerebellum was increased 1 hour after DDT administration, which was before amplitude of the electrical activity of the vermis was increased and before tremoring was grossly evident 4-13 hours after DDT. Amplitudes of electrical activities were maximal in the vermis and neocerebellum at 4 to 7 hours. High amplitude spiking was still evident in the vermis at 9 and 13 hours, whereas activity in the neocerebellum at these times was largely restored toward normal. During the periods of maximal increase in electrical activity, spikes appeared synchronously in the vermis and neocerebellum. This study showed that cerebellar SEA was increased before gross behavioral changes, such as tremoring, were evident, so that increased amplitude of cerebellar SEA was another sensitive index of DDT action.

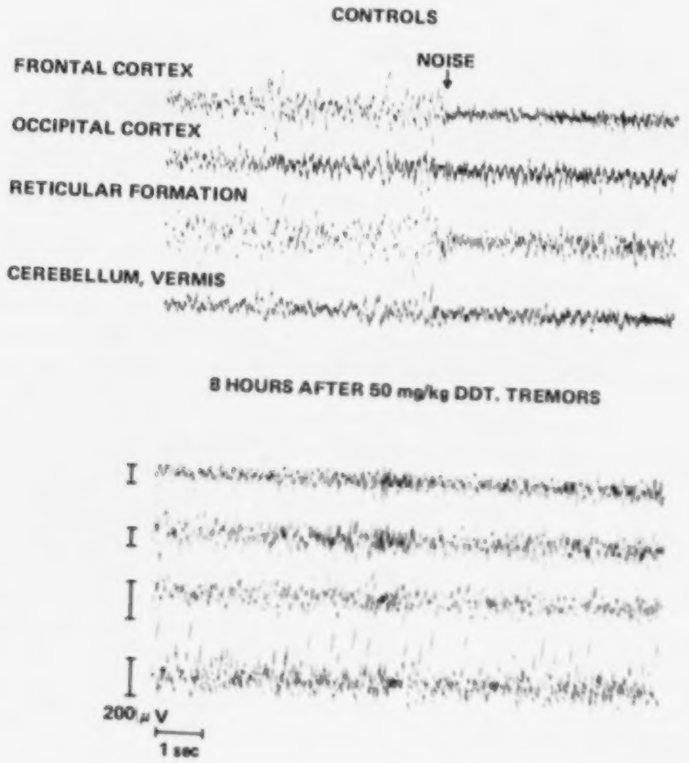


Figure 9-6. The spontaneous electrical activity of 4 brain areas before and 8 hours after DDT *per os* in the rat. A sudden noise during the control recording session changed the patterns of the SEA from those typical of inattention to those typical of arousal. After DDT administration arousal patterns prevailed and "spiking" occurred in cerebellar electrical activity even in the absence of external "arousing" stimuli.

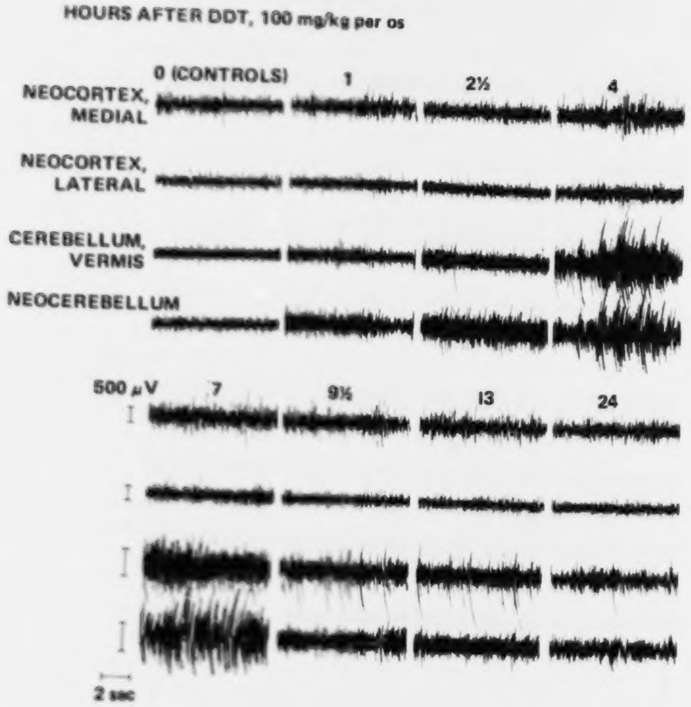


Figure 9-7. Comparison of the changes in electrical activity of 2 cerebral cortical (somesthetic) areas and 2 cerebellar areas produced by DDT administration. Amplitude of electrical activity increased in the neocerebellum sooner than in the vermis. Amplitudes of both cerebellar electrical activities increased more than cortical activities at the height of the effect of DDT. Spiking continued in the vermis for a longer period of time than in the neocerebellum during recovery.

The principal conclusions which may be drawn from studies presented thus far illustrate some of the advantages and limitations of electrophysiological studies in toxicology. The studies demonstrated that after DDT administration in the awake, unrestrained animal: (1) changes in spontaneous brain electrical activity may be detected before changes in spontaneous behavior; (2) the electrical activity of some brain areas changes before and to a greater extent than that of other areas; (3) changes in the respiratory waves in the electrical activity of the olfactory system show that low levels of DDT stimulate respiration and that this is a sensitive, early index of DDT action; (4) marked increases in the arousal waves of the olfactory system and in the arousal patterns of other brain areas show that low levels of DDT are strongly excitatory to the CNS, in agreement with the behavioral hyperexcitability caused by DDT; and (5) the greatest changes in brain electrical activity occur in the cerebellum.

Effects of DDT on Evoked Potentials

The striking effects of DDT in increasing the electrical activity of the cerebellum suggest that DDT has a direct stimulatory effect on the cerebellum. Another possibility is that the increase in cerebellar electrical activity merely reflects the greater proprioceptive input to the cerebellum because of increased muscle activity during tremors. Therefore, the effect of DDT on visual and auditory potentials in the cerebellum and other brain areas were investigated. A greater effect of DDT on potentials evoked by light and sound in the cerebellum than in other brain areas would be compatible with the hypothesis that DDT preferentially affects the cerebellum.

Auditory potentials were evoked by a 1,000 Hz tone of 30 msec duration and 60 db above threshold as judged by oscilloscope tracings. Light flashes (10 μsec duration, maximal intensity, 18 inches above the animals) for visual stimulation were produced with a Grass photostimulator (model PS-2). Responses of various brain areas were amplified and averaged with a Northern Scientific online computer (model No. 544). Stimulation rate was 1/sec and 50 responses were summed to produce each averaged evoked potential.

Figure 9-8 shows the typical effects of 100 mg/kg of DDT on auditory evoked potentials in cerebral cortex, midbrain reticular formation, and vermis of the cerebellum. In frontal and occipital cortex the responses were depressed at the height of the effects of DDT which occurred 6 to 12 hours after administration. On the other hand, some components of both visual and auditory responses in the cerebellum and reticular formation were significantly increased in amplitude. Early components of the cerebellar response, which were often not present during the control period, appeared after DDT administration, increased in amplitude during the maximal effects of DDT, and then disappeared again during the recovery period (Figure 9-8).

The depression by DDT of the evoked responses in the cerebral cortex may be explained by a "line-busy" effect, i.e., that the increased spontaneous or background electrical activity caused by DDT interfered with the neuronal response to the light or sound stimulus. On the other hand, the effect of DDT on the cerebellum was unique because both spontaneous and evoked electrical activities were increased.

The effects of DDT were compared with those of tremorine, which is also a tremorigenic agent. Responses evoked by sound recorded at several positions in the cerebellum before and after administration of tremorine clearly showed that sound-evoked cerebellar potentials were completely eliminated by tremorine, in marked contrast to the effect of DDT in increasing amplitudes of these potentials in the cerebellum. Behaviorally, tremorine produced an absence of the startle response, the rats appearing to be almost oblivious to visual and auditory stimuli, in marked contrast to the effects of DDT in increasing the startle response.

The relation between the potentiation of the startle response and visual and auditory responses in the cerebellum, as produced by DDT, and the elimination of the startle response and visual and auditory cerebellar responses, as produced by tremorine, may be more than coincidental. It has been reported that

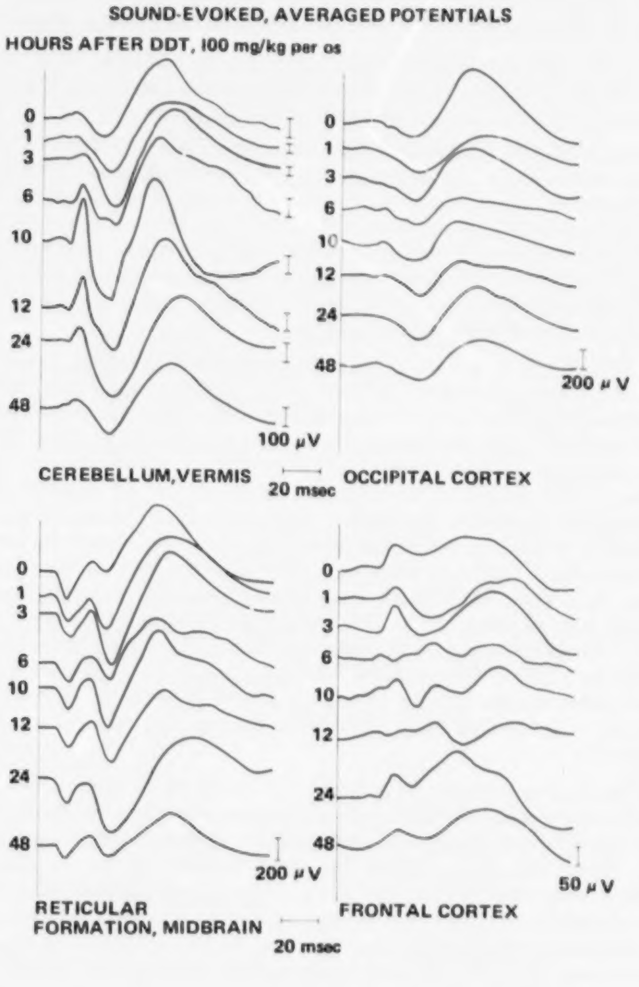


Figure 9-8. Effects of a single dose of DDT on averaged auditory evoked potentials recorded simultaneously from four different brain areas in the awake, unrestrained rat. Note the changing scale for amplitude of cerebellar evoked potentials. Amplitudes of the responses recorded from the frontal and occipital cortex were depressed by DDT, especially 6 hours post administration, whereas some components of the responses evoked in the cerebellum and reticular formation increased in amplitude. The major effect of DDT was to cause the appearance and increase the amplitude of an early component of the response recorded from the cerebellum. This early response was first evident 6 hours after DDT, reached maximal amplitude at 10 hours, and had disappeared by 48 hours post administration.

lesions in portions of the vermis, which also receive visual and auditory inputs, result in absence of the startle response (Chambers and Sprague, 1955).

DDT and tremorine produced similar increases in cerebellar SEA. These increases are attributed to increased proprioceptive input to the cerebellum resulting from tremoring and increased muscle activity.

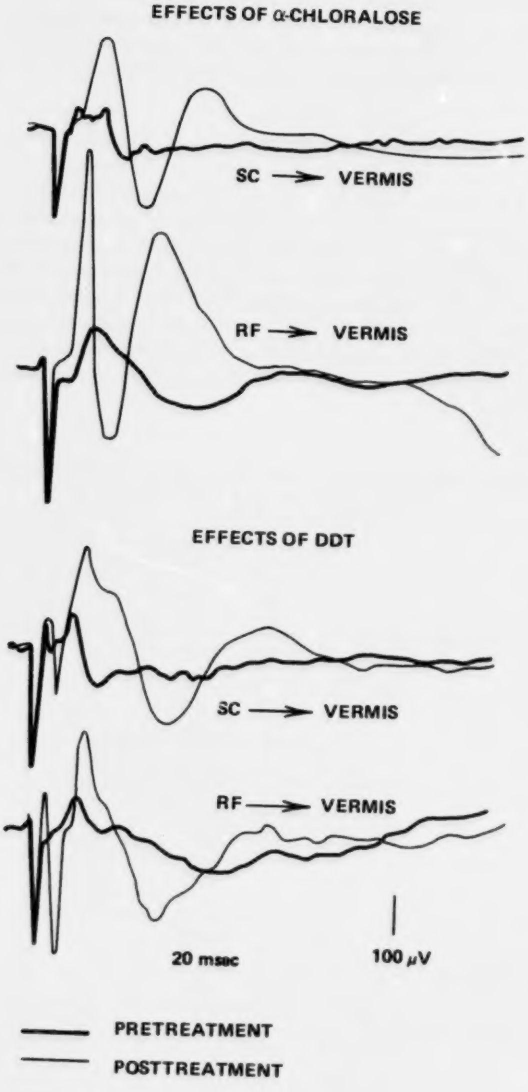
Another drug which has been shown to increase visual and auditory responses in the cerebellum, at least in the cat, is α-chloralose (Fadiga and Pupilla, 1964). Amplitudes of visual responses recorded from several electrode positions in the cerebellum were clearly increased by the drug. This is particularly interesting because α-chloralose also caused an exaggerated startle response to sudden loud noises. This lends further support to the hypothesis that DDT increases the startle response by an action on the cerebellum, and that the cerebellum is basically related to the startle response.

The pathways by which visual and auditory potentials reach the cerebellum are not completely understood. However, there is evidence that these teleceptive impulses relay in the tectum (superior and inferior colliculi) and in the brainstem enroute to the cerebellum (Fadiga and Pupilla, 1964). The next study then undertook to investigate the effect of DDT and α-chloralose on responses evoked in the cerebellum by electrical stimulation of the superior colliculus and reticular formation (Figure 9-9). During the control period it was almost impossible to elicit anything except a low-amplitude response in the cerebellum by stimulating this way. However, after administration of either chemical agent, the cerebellar response was increased many-fold. Additional studies showed that the responses to light and sound in the superior colliculus and reticular formation were not themselves potentiated by DDT. Thus, the potentiation by DDT of the cerebellar response to electrical stimulation of the superior colliculus and reticular formation appears to be due to a direct effect on the cerebellum or on a brain area relaying the impulses from the lectum to the cerebellum.

The studies presented so far represent an effort to understand the types of effects produced on the brain by acute exposure to DDT. An important question was whether or not DDT preferentially affected some brain areas more than others, and, if so, whether this could be related to the symptomatology of DDT poisoning. The evoked potential studies provide evidence that DDT in sublethal doses affects the cerebellum particularly, that this effect is one of increased excitability, and that this effect on the cerebellum may be a factor contributing to the exaggerated startle response characteristic of DDT poisoning. Comparison of the effects of DDT with those of drugs which produce some of the clinical symptoms also produced by DDT helped delineate some of the rather unique features of the effects of DDT on the brain.

Distribution of DDT in Brain and Spinal Cord

Next, the concentrations of DDT in several brain areas and spinal cord of the rat were determined at various times after a single dose of 100 mg/kg *per os* of DDT dissolved in oil (Table 9-1; Woolley and Runnells, 1967). The hope was that a correlation between symptomatology and CNS DDT concentrations


EFFECTS OF α-CHLORALOSE

EFFECTS OF DDT

Figure 9-9. Effects of alpha-chloralose and DDT on responses evoked in the cerebellar vermis by electrical stimulation of the superior colliculus (SC) and midbrain reticular formation (RF). Both chemical agents potentiated the responses recorded in the cerebellum.

could be established. Because of the well-known lipid solubility of DDT, it was of interest to determine the pattern of uptake and release of DDT in a sample of neocortex, which is gray matter only, and the pattern in brainstem (medulla and pons) and spinal cord, which have a high percent of white matter and thus of lipid.

When DDT concentrations were expressed in terms of wet tissue weight (Table 9-1), concentrations were 40 percent higher in the neocortex than in the spinal cord at 6 hours. However, at 12 and 24 hours DDT concentrations were significantly higher in brainstem and spinal cord than in neocortex and cerebellum, suggesting that CNS areas with high lipid (i.e., high myelin) concentrations took up DDT more slowly but retained it longer than did CNS areas which were primarily gray matter only. When DDT concentrations were calculated for the whole brain and compared with spinal cord concentrations, it was evident that DDT concentrations per unit fresh weight were about the same at 6 and 12 hours but were about twice as high in the spinal cord as in the brain at 24 hours.

Table 9-1. DDT CONCENTRATIONS IN TISSUES OF THE RAT WITH TIME AFTER A SINGLE DOSE OF DDT (100 mg/kg *per os*)

Tissue	DDT concentration (µg/g fresh tissue) Hours after DDT administration 6	12	24	% Lipid in fresh tissue
Neocortex	35.2[a] ±4.0	20.2 ± 2.5	13.1 ±1.7	6.0 ±0.5
Cerebellum	29.0 ±3.1	20.9 ±2.3	13.6 ±1.5	6.8 ±0.5
Brainstem	27.2 ±2.2	34.9 ±4.2	17.5 ±1.9	16.1 ±0.7
Remaining brain	20.0 ±1.9	23.2 ±2.7	11.2 ±0.5	7.2 ±0.4
Whole brain	22.0 ±2.4	22.2 ±2.5	11.8 ±0.5	7.2 ±0.3
Spinal cord	24.5 ±2.0	27.2 ±2.8	25.1 ±3.1	17.9 ±0.6
Liver	216 ±17	165 ±9	43 ±5	4.2 ±0.6
Plasma	±5.9 ±0.9	3.2 ±0.3	3.4 ±0.4	– –

[a] Mean ±SE for 8 rats per group. From Woolley and Runnells, 1967.

In these animals, hyperirritability and tremor, the outstanding symptoms produced by the dose of DDT used, were generally more intense at 6 than at 12 hours and so appeared to correlate with the peak concentrations in the neocortex and cerebellum. However, because the time periods selected were so widely spaced, this correlation cannot be made with certainty. According to the literature, reviewed by Hayes (1959) and Shankland (1964), during DDT poisoning in the intact animal, a fine tremor is present initially and is eventually replaced by a coarse, low frequency, tremor. In spinal animals the tremor is usually coarser in the body parts innervated by the spinal cord caudal to the lesion than it is in the intact animal. The fine tremor may occur when DDT effects on gray matter in brain predominate, whereas the coarse tremor may be indicative of predominant effects on the spinal cord.

Effects of Parathion

Parathion is a widely used, highly toxic organophosphate insecticide, which is oxidatively desulfurated *in vivo* to its active metabolite, paraoxon. Paraoxon is a powerful acetylcholinesterase (AChE) inhibitor and so is believed to affect cholinergic synapses or junctions by reducing the rate of breakdown of acetylcholine (ACh), which in turn, permits buildup of excess ACh.

The effects of parathion administration (3 mg/kg sc) on brain electrical activity were determined in six female Sprague-Dawley rats with chronically implanted brain electrodes (Woolley, 1976). Visual and auditory evoked potentials were recorded in the visual cortex, superior colliculus, midbrain reticular formation, and cerebellum. Potentials were elicited and averaged as described above.

The typical effects of parathion on visual evoked potentials recorded in the superior colliculus (Figure 9-10) and the visual cortex were to increase latency, i.e., the time from the flash until the beginning of the response, and to decrease amplitude of the waves of the response. Latency was increased 40 percent or more in responses recorded from the visual cortex and superior colliculus, whereas latency of responses recorded simultaneously in the cerebellum and reticular formation were affected less. Amplitudes of the main components of the responses of the visual cortex and superior colliculus were reduced to one-third or less of control amplitudes. Amplitudes of the visual evoked responses recorded in the cerebellum and reticular formation also tended to be reduced, but variability was greater for these responses than for those recorded in the cortex and colliculus. Effects on amplitude and latency were usually marked 2 and 4 hours after parathion administration, although they were sometimes pronounced as early as 45 minutes after administration. The responses had returned to pretreatment values by 8 hours after parathion administration.

The effects of parathion administration on sound-evoked potentials in the reticular formation (Figure 9-11) were to decrease markedly the amplitude 2 and 4 hours after administration with some recovery at 8 and 20 hours. In contrast to effects on visual evoked potentials (Figure 9-10), marked changes in latency did not occur in auditory evoked potentials. Thus, the effects of parathion administration on auditory evoked potentials were less marked than on visual evoked potentials.

These observations permit two conclusions: (1) there must be a cholinergic link somewhere in the generation of these visual and auditory evoked potentials, and (2) recovery of visual evoked potentials from the effects of parathion administration is surprisingly fast when one considers that paraoxon is believed to produce irreversible, or at least only slowly reversible, inhibition of AChE activity (O'Brien, 1967).

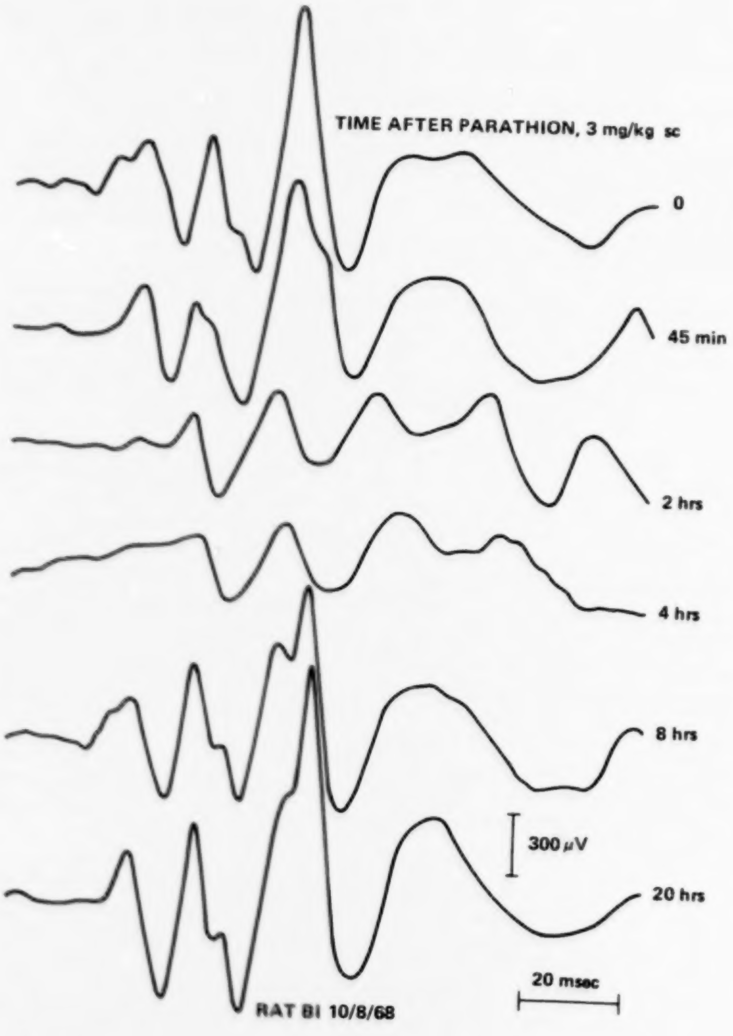


Figure 9-10. Effects of parathion on averaged flash-evoked potentials in the superior colliculus of the adult female rat. Latency was increased and amplitude of the major component was decreased 2 and 4 hours after administration of parathion. Recovery was evident at 8 hours.

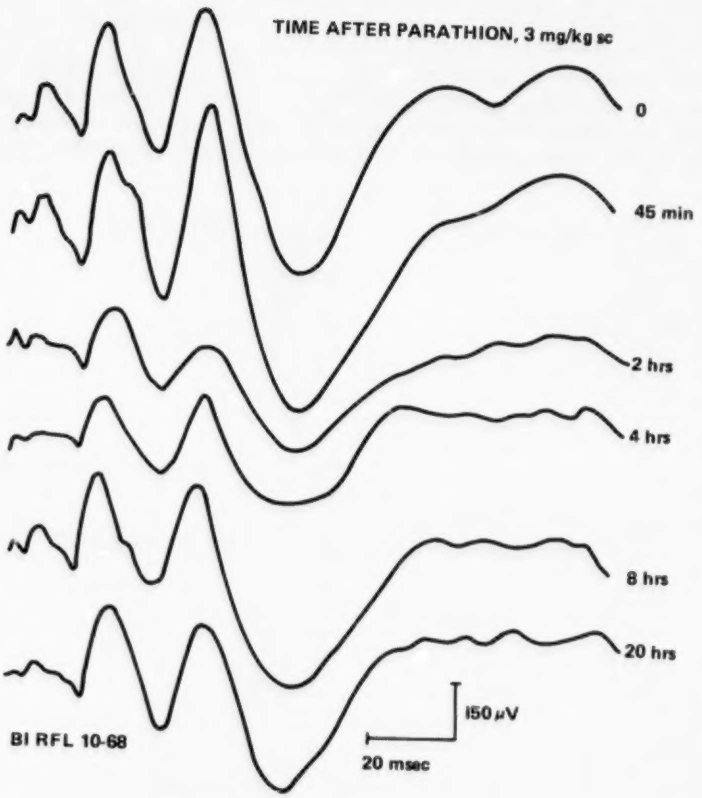


Figure 9-11. Effects of parathion administration on averaged sound-evoked potentials in the midbrain reticular formation of the adult female rat. Amplitude was decreased at 2 and 4 hours post parathion but latency was not altered markedly.

Regarding the first point, histological evidence for involvement of cholinergic synapses in generation of visual evoked potentials has been provided by Shute and Lewis (1967), who have described AChE-containing fibers rising from the midbrain and passing via the dorsal and ventral tegmental pathways directly and indirectly to the anterior and posterior colliculi, lateral and medial geniculate bodies, pretectal area, and cerebral cortex.

Some controversy exists concerning the rate of recovery of brain AChE activity after parathion adminstration in the rat. Although an early report described recovery of brain AChE activity 4 hours after administration of parathion in the rat (Du Bois *et al.*, 1949), another stated that several days are required (Davison, 1953), and still another that a month is required for recovery

(Giachetti *et al.*, 1966). Therefore, we determined the time-course of inhibition and recovery of AChE activity after intraperitoneal (ip) and subcutaneous (sc) administration of 2 mg/kg parathion in the adult female rat. The colorimetric method of Ellman *et al.* (1961) was used to measure AChE and ChE (pseudocholinesterase) activities. The results (Figure 9-12) showed that blood AChE and plasma ChE activities recovered rather rapidly, i.e., within the 1 week of this study. However, brain and spinal cord AChE activities reached

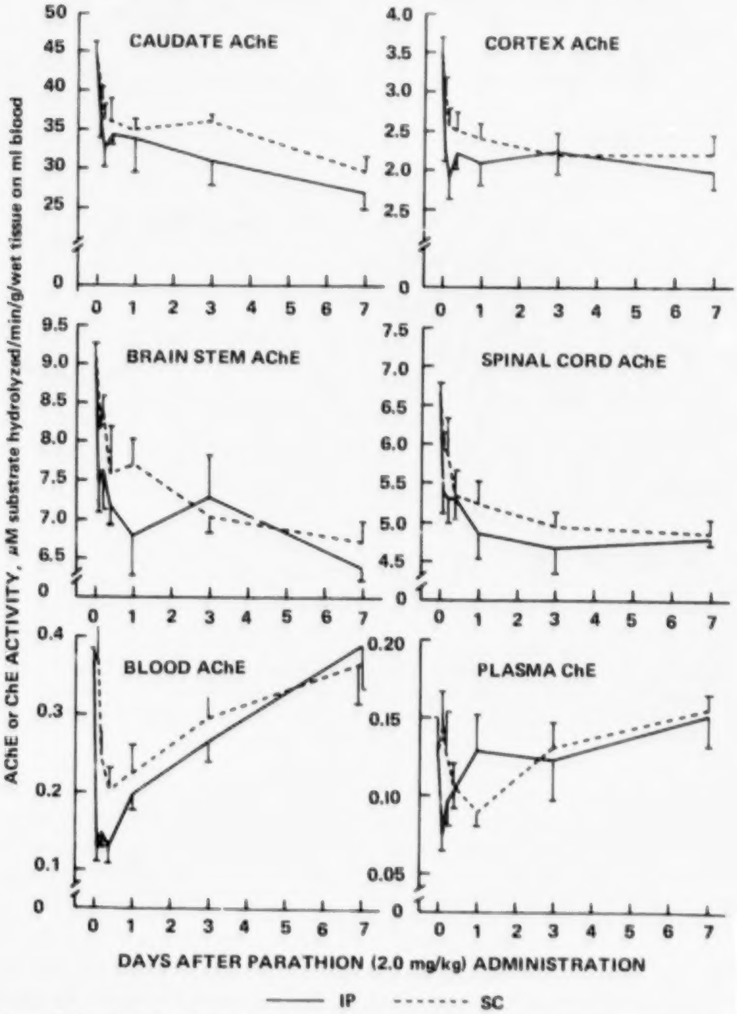


Figure 9-12. Effects of ip and sc administration of parathion in the rat on AChE activities in brain, spinal cord and blood and on ChE activity in plasma. Each point represents the mean, and the vertical bracketed lines represent the standard errors about the mean for 6 animals in this and the following figure.

peak inhibition about 9 hours after parathion administration and showed essentially no recovery during the study. When the experiment was repeated and recovery followed over a longer time period (Figure 9-13), it was again observed that blood AChE and plasma ChE activities had recovered by 1 week after ip administration of parathion (2.25 mg/kg). AChE activities did not become inhibited as rapidly in brain as in blood and required a longer time to recover in brain than in blood. Inhibition of AChE activities in caudate, cerebral cortex, and brainstem was greater at 1 week than at 6 hours post parathion administration; recovery was complete between 2 and 4 weeks after administration of parathion.

From these results it is evident that the effect of parathion administration on visual evoked potentials disappeared despite continuing inhibition of AChE

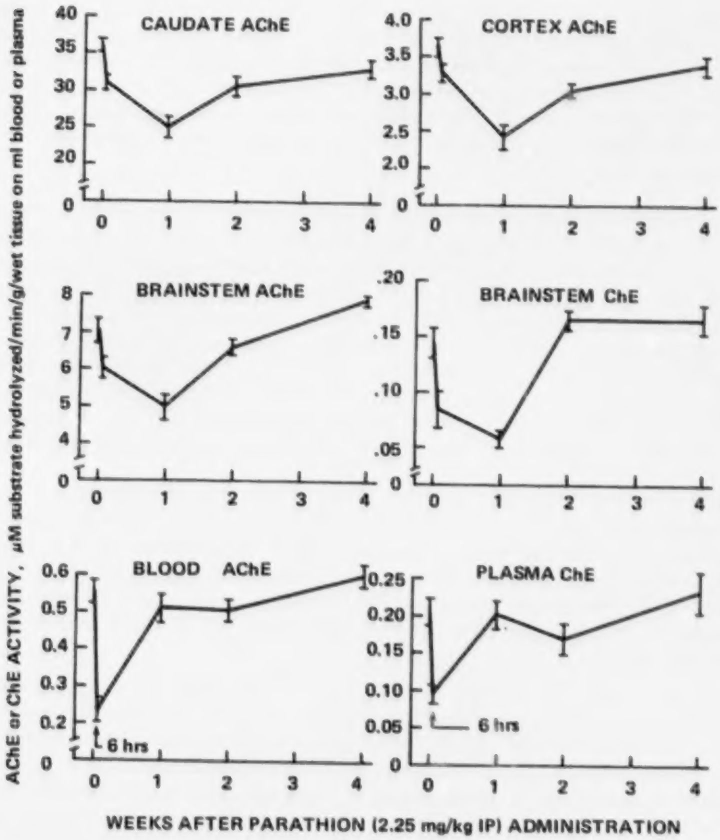


Figure 9-13. Effects of ip administration of parathion on inhibition and recovery of AChE and ChE activities in various brain areas, blood or plasma of the rat.

activity in the rat. Visual evoked potentials recovered within 8 hours after parathion administration, but blood AChE activity required about a week to recover, and brain AChE activities required 2-4 weeks for recovery. Effects on evoked potentials were maximal when CNS AChE activities were falling, and these effects disappeared after AChE inhibition had reached its maximum and stabilized.

One interpretation of these findings is that development of adaption or tolerance to depression of AChE activity in the CNS occurred after parathion administration in rats and monkeys. The problem of tolerance in relation to a number of anticholinesterase agents has been reviewed recently (Bignami *et al.,* 1975). Usually, an AChE inhibitor is administered and cholinergic symptoms are immediately evident, but despite continued AChE inhibition, the symptoms soon disappear. The major present hypotheses regarding the mechanism of onset of tolerance to AChE inhibition are as follows: (1) ACh levels, which become elevated immediately after AChE inhibition, become lower with time despite the persisting depression of AChE activity; (2) prolonged exposure to elevated ACh levels brings about a reduction in receptor sensitivity, thus counteracting the effects of AChE inhibition and allowing a recovery of function; and (3) the activity of other neurophysiological systems, either agonistic or antagonistic, alters to counteract the overstimulation of cholinergic systems.

Still other interpretations of the dissociation between the neurological effects and AChE inhibition after parathion administration remain to be explored. AChE and the ACh receptor must share some structural similarities because both combine with ACh. Paraoxon also combines with AChE at the active esteratic site and therefore probably also has the structural requirements to react with the ACh receptor. It is possible that paraoxon first reacts with AChE to inhibit it and thus to permit a buildup of ACh at cholinergic synapses. After some delay paraoxon may next combine with the ACh receptor and in this way reduce receptor sensitivity to the elevated ACh levels and permit a return to more normal functioning, even though AChE activity is strongly inhibited.

Still another explanation has been provided recently by observations that synaptosomes prepared from rats shortly after injection of a single dose of paraoxon showed significantly increased release and synthesis of ACh. However, when a slightly smaller dose of paraoxon was injected once daily for five days for an even greater total injection, the rate of synaptosomal synthesis and release of ACh did not differ from controls even though AChE activity was strongly inhibited.

This may mean that paraoxon initially acts on synaptosomes to increase ACh release and synthesis, thus causing cholinergic symptoms, but that the synaptosomes adapt to this effect of paraoxon, and so the cholinergic symptoms disappear (Speth *et al.,* 1975). If further work substantiates this new finding, it would suggest that the development of "tolerance" to the AChE inhibiting effects of parathion administration is not an actual tolerance but merely reflects the fact that the mechanism of action is primarily on neurotransmitter release and that AChE inhibition is of lesser importance in the symptomatology of parathion poisoning, at least at low doses of parathion.

The Value of a Multifaceted Approach in Neurotoxicology

A strong argument for using a multifaceted approach for investigating the effects of a toxicant is that an investigator may simply be misled regarding the intensity, time, and duration of action of a toxicant if he considers only one endpoint. Each of the various methods for assessing neural function has its advantages and limitations. Behavioral tests are usually very sensitive in their ability to detect the effects of low levels of toxins. A major reason for this sensitivity also accounts for one of the more frustrating aspects of behavioral testing. In behavioral tests the animal is frequently treated like a "black box." An input of some sort is applied to this "box," and the output, behavior, is measured. Modifications in behavior may be produced by toxins or other agents because of changes in any one or more of the many links in the neural chain of events between input and output or by changes in non-neural systems which secondarily alter the nervous system. The result is that the behavioralist may be left with the satisfying feeling of having recorded a change in behavior but with the frustrating feeling of not knowing why the change has occurred. To determine "why," it is usually necessary to perform additional tests. These other tests may involve electrophysiological techniques or biochemical procedures.

In a clinical setting it is frequently easier to measure some biochemical endpoint, e.g., blood AChE activity, than to conduct extensive behavioral or electrophysiological testing. Hence, experimental analyses for relevant biochemical changes or for levels of a toxin or its metabolites should be carried out in readily obtainable tissues such as blood, urine, or hair, along with behavioral or electrophysiological testing. From the data thus obtained, it may be possible to predict biochemical measurements which may be carried out clinically to provide a warning that nervous sytem functioning may be impaired at a certain level of change in tissue components. Naturally, there may be pitfalls to such an approach. In the study reported here, the early effects of parathion poisoning on brain evoked potentials were correlated with falling levels of blood AChE activity but not with the absolute level of AChE activity.

The principal advantage of electrophysiological testing, using animals with chronically implanted brain electrodes and recording while the animals are awake, unanesthetized, and relatively unrestrained, is that the differential effects of a toxicant on various brain areas or brain systems may be investigated. This, in turn, should lead to a better understanding of the mechanism of action of the toxicant. Using these procedures in previous work in this laboratory, it was possible to demonstrate the selective activation of the cerebellum and olfactory brain areas by DDT (Woolley and Barron, 1968). It was also demonstrated that parathion administration had greater effects on visual evoked than on auditory evoked potentials in rats with chronically implanted brain electrodes and that there was a dissociation between the time course of effects of parathion administration on evoked potentials and on brain AChE activities. Electrophysiological techniques may be more sensitive than some other endpoints to the effects of a toxin. Changes in spontaneous brain electrical activity after DDT were evident before changes in spontaneous behavior, for example. Brain electrical activity may be recorded continuously before, during, and after administration of a toxin, so that each animal may serve as its own control.

Finally, however, an obvious limitation of electrophysiological techniques is that the investigator must have specialized equipment and training to be able to record and interpret electrophysiological data. Perhaps, this is the reason for the relative rarity of "electrotoxicologists."

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10. NEUROBEHAVIORAL TOXICOLOGY: PROBLEMS AND METHODS IN HUMAN RESEARCH

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Other papers in this volume describe methods for studying the effects of toxicants on the behavior of species ranging from mice to monkeys and sheep. Research on the human species, however, imposes a complex set of contraints, both ethical and empirical, which essentially limit the investigator to noninvasive procedures. This chapter will review these constraints and describe a useful method for studying the behavioral and electrophysiological effects of toxicant exposure in man.

The effect of chemical insult on central nervous system (CNS) function has often been inferred from behavioral measures such as visual threshold (McFarland *et al.,* 1944) or temporal discrimination (Beard and Wertheim, 1967). Direct measures of CNS function are ultimately required to validate behavioral inferences and to elaborate the neurophysiological effects of toxicant exposure on brain tissue. Electrocortical events recordable from the scalp of humans provide a noninvasive measure of electrical activity of the brain. If these events are recorded during the performance of sensorimotor or cognitive tasks, changes in behavior and CNS function can be measured concurrently. Moreover, neuroelectric events can be recorded from the scalp or from electrodes implanted in specific brain structures (cf. Woolley, this volume). Event-related potentials of the brain thus offer a promising bridge for the comparative study of neurobehavioral toxicant effects in man and animal.

Methodological Problems in Human Research

Individual Differences

Philosophers have devoted volumes to the concept of free will, or the tendency of man to behave in a nondeterministic, unpredictable manner. In the laboratory this translates to individual differences and intersubject variability. If free will is the unique characteristic which differentiate man from beast, it poses a great nuisance in behavioral toxicology. Application of stringent criteria based on standard personality and medical inventories, physicals, and interviews to secure "normal, healthy" populations has been remarkably unsuccessful in reducing intersubject variability. No matter how rigorously subjects are screened in preparation for human experiments, there is no hope of ever attaining a pure laboratory strain of *Homo sapiens.*

This problem is particularly severe when the objective is to define the *threshold* level at which a given toxicant produces a *significant impairment* in function. Threshold effects, by definition, are "just noticeable differences."

How can individual variability be minimized in the study of toxicant thresholds? The standard approach is to use a repeated-measures design in which each subject is exposed double-blind to multiple levels of a toxicant, including a "no drug" control or baseline condition. The variability attributable to individual differences can then be partialled out by subtracting control values from exposure values. The residual, theoretically, represents the effect of toxicant exposure. One disadvantage of the repeated-measures design, compared to an independent-measures design, is a loss of degrees of freedom and, therefore, a loss in statistical power. Repeated-measures designs also place restrictions on exposure regimens due to "carry-over" effects, a problem which will be discussed later.

Phylogenetic Differences in Brain Function

Language Capacity

One of the complaints voiced frequently by behavioral toxicologists is the length of time required to train a rat or monkey to perform a simple discrimination. *Homo sapiens,* with his advanced language capacity, can be handed a written set of instructions instead of patiently shaping his behavior with operant conditioning techniques. The training period is certainly shorter in man, but the advantage gained is often illusory.

In communicating verbally or in writing, the investigator is faced in human research with a dismaying problem called "instructional set." Instuctions must be carefully standardized across subjects. The unique blend of expectancies, motivation, and experience which each subject brings to the laboratory has considerable bearing on how the individual responds to instuctions. More than likely, the subject will be a college student, peering skeptically at the instructions and trying to second-guess the investigator.

Cortical Development

Language capacity is an example of cortical development which differentiates *Homo sapiens* on the phylogenetic scale. Man has a proportionately larger cortex, particularly in the frontal region, than most animals. This capacity permits the study of more complex "cognitive" tasks than might be considered with other species. Again, the advantages are probably illusory, since complex tasks yield complex results which may obscure the simple effect of toxicant exposure.

The extensive CO studies of Beard and Grandstaff (1975) provide a good illustration. Impairments in performance due to CO exposure were observed in simple visual and auditory signal detection tasks, but when subjects were required to make a complex spatial rotation, no CO effects were observed. Beard and Grandstaff concluded that the physiological arousal produced by the complex, challenging task completely compensated for the subtle impairment observed during CO exposure in other simple, monotonous tasks.

Sensory Dominance

Another dimension of brain function which varies widely among species is sensory specialization. Olfaction is the dominant modality in rats, while vision and hearing are dominant in man. Differences in sensory dominance complicate interspecies comparisons and must be taken into account in experimental design. Sensory discrimination tasks appropriate for one species may not be appropriate for another. Cats, for instance, have very poor color vision, while primates have excellent color vision. Unless the toxicant under study is known to have a modality-specific effect, it would seem prudent to challenge a dominant, rather than a minor sensory function.

In practice, the investigator frequently does not know which sensory modality, if any, will be sensitive to toxicant exposure. A good strategy is to challenge multiple modalities to determine comparative sensitivities. Misconceptions can easily arise if observation is limited to a single modality, particularly when the results are negative. Dyer and Annau (in press), for instance, failed to find any low level CO effect on visual evoked potentials in the rat. Groll-Knapp *et al.* (in press), however, compared the effects of low level CO on somatosensory (SEP), auditory (AEP), and visual evoked potentials (VEP) in humans. The SEP was maximally sensitive, the AEP was less sensitive, and the VEP was *insensitive* to CO effects. Although it is risky to extrapolate across species, the significance of Dyer and Annau's negative findings is difficult to evaluate without comparative evidence from other sensory modalities in the rat.

Ethical Considerations

Federal Requirements

Some scientists would prefer to leave ethical considerations to philosophers or theologians, but the federal government has legislated otherwise in the case of human research. The National Research Act of 1975 (PL 93-348) established guidelines to safeguard the rights and welfare of human subjects. The Department of Health, Education, and Welfare has assumed primary responsibility for implementation and enforcement of PL 93-348 in accordance with the Code of Federal Regulations (45CFR46). DHEW-supported activities conducting human research are required to establish an Institutional Review Board to assure the protection of human subjects. Investigators must obtain approval from the Board before initiating any study of human subjects and must submit periodic reports to the Board to assure compliance with federal guidelines.

Informed consent must be obtained from subjects (or legally authorized representatives in the case of minors, prisoners, or individuals who are mentally or physically incompetent). To illustrate the stringency of requirements, the following information must be provided to satisfy the definition of "informed consent" (45CFR§46.103):

- A fair explanation of the procedures to be followed and their purposes, including identification of any procedures which are experimental;
- A description of any attendant discomforts and risks reasonably to be expected;
- A description of any benefits reasonably to be expected;
- A disclosure of any appropriate alternative procedures that might be advantageous for the subject;
- An offer to answer any inquiries concerning the procedures; and
- An instruction that the person is free to withdraw his consent and to discontinue participation in the project or activity at any time without prejudice to the subject.

Federal agencies engaged in human research are subject to further constraints on the type of information that may be collected (Privacy Act of 1974) and the manner in which the information may be disseminated (Freedom of Information Act of 1966). The objectives of these regulations are laudable, although the practical results are increased paperwork and slower implementation of research.

Exposure Limits

Ethical considerations limit the study of toxicant effects to relatively low exposure levels in adult populations. Therefore, only the lower segment of a dose-response curve can be experimentally defined in humans, although accidental or occupational exposures may supplement data obtained in the laboratory.

The usual objective in studying the effects of low concentrations is to determine the threshold toxicologic dose (TTD) or "no effect" level. The TTD may be defined as the concentration below which no functional impairment can be demonstrated. In principle, experimental procedures to determine TTD and LD_{50} are similar, i.e., methods to estimate the dose at which 50 percent of the true population will exhibit a specified effect within prescribed statistical confidence limits. Death is an absolute, unambiguous criterion of effect in determining the LD_{50}. Functional impairment, on the other hand, is the relatively ambiguous criterion of effect in determining TTD. This criterion is operationally defined relative to performance in a nonexposed control population.

If an overt behavioral parameter—such as the error rate in a vigilance task— is chosen as the criterion function, then the *physiological* significance of any observed functional impairment must be *inferred* from behavior. For example, what is the physiological significance of an increase in the number of visual targets missed during exposure to 75 ppm carbon monoxide compared to clean air? Although the observed effect may be significant at the 0.05 level of confidence, behavioral, statistical, and physiological significance are not synonymous.

Furthermore, the toxicologic threshold is a function of methodological sensitivity as well as absolute chemical potency. That is, the absence of an observed effect may be due to the use of an inappropriate index or a crude measurement device which is incapable of detecting an actual toxicologic effect. The choice of an appropriate test assumes that the investigator knows in advance which function is most likely to be affected. With new drugs or untested toxicologic substances, this assumption is frequently untenable. In this case, preliminary study of multiple functions must be undertaken to identify which is most sensitive to the substance under investigation. When the appropriate function has been identified, the investigator must then select the most sensitive measurement available. In the behavioral sciences, this choice is problematic since there are as many different tests as there are pine trees in Georgia. The need for standardized, properly-validated test batteries is critical in behavioral toxicology and in the behavioral sciences in general.

The investigator is limited in the *duration* as well as the level of toxicologic exposure in human research. Economic, social, and ethical considerations limit the length of time that humans can be confined in an experimental chamber. Human studies generally consist of short-term exposures lasting no more than 2-4 hours. Repeated acute exposures may be made at periodic intervals of days, weeks, or months. Between exposures, however, the subject generally departs from the laboratory and the sphere of experimental control.

Acute high-level exposures occur occasionally in humans as a result of accidents or natural disasters. Chronic low-level exposures are also encountered in occupational environments. The investigator has no control over such exposures, and the conclusions which can be drawn from these isolated instances are limited. Therefore, chronic, *in utero,* and longitudinal toxicologic studies must be undertaken in species other than *Homo sapiens.*

Noninvasive Measures

Human research in normal adult populations is limited to measurements which do not require any major surgical intervention. If there is no added risk to patient health, basic researchers can often collaborate with physicians treating patients with acute disorders which entail surgical intervention. Penfield and Jasper (1954), for instance, contributed greatly to the knowledge of neurophysiology by electrically stimulating the cortex of patients undergoing stereotaxic craniotomy for treatment of intractible epilepsy. This kind of fruitful collaboration, however, is rarely available to the behavioral toxicologist except in the case of accidental poisoning. Thus, systematic studies of physiological parameters which require implantation of cannulae of electrodes must be undertaken with nonhumans.

Sex Differences

The variable of sex is often ignored in human research. The potential danger to an unborn fetus, however, precludes the use of pregnant women in toxicological research. Nor can the investigator rely on verbal report, since a prospective subject may not know if she is pregnant. Women should be given pregnancy tests before exposure.

Menstrual cycles also pose a problem which must be taken into account, particularly if the experiment requires repeated exposures at fixed intervals (e.g. weekly). Since the behavioral effects of toxicants, particularly at low concentrations, tend to be extremely subtle, the physiological and psychological states of subjects must be maintained as constant as possible.

In view of these problems, the simplest recourse is to limit human toxicological research to male subjects. This alternative can pose problems in the present climate of sexual egalitarianism. For instance, three young ladies walked into our laboratories recently, demanding equal time and equal pay. When they were shown what the experiment would entail—pedaling a bicycle ergometer in a transparent plastic chamber while stripped to the waist for ECG recording—two blushed and left the laboratory. The third, however, smiled and asked when she could begin. A practical difficulty in accommodating such egalitarianism is that sample size must be doubled to control for sex differences.

Experimental Control in Human Research

Many variables such as diet, length and pattern of sleep, and learning history can be easily controlled in animal populations. These variables are extremely difficult to control in human subjects who come into the laboratory for short-term acute exposures. Even with elaborate instructions and questionnaires, it is impossible to control or determine unequivocally what subjects do outside the laboratory.

One approach to this problem is to continously monitor the behavioral and physiological state of subjects for an extended period of time before, during, and after exposure. Telemetry systems offer a flexible tool for remote monitoring of free-roving subjects in work or home environments. Live-in facilities which permit continuous, intensive study for periods of days or even weeks, however, provide the most effective control of variables such as diet, sleep, exercise, and toxicant exposure.

The Clinical Laboratory Evaluation and Assessment of Noxious Substances (CLEANS) facility in Chapel Hill, North Carolina provides an example of live-in chambers designed specifically for studying the health effects of environmental toxicants in humans. The CLEANS facility consists of two large exposure chambers with a computerized physiological data acquisition system as diagrammed in Figure 10-1. Up to four subjects can be studied simultaneously in each chamber, permitting rapid collection of data for characterizing the

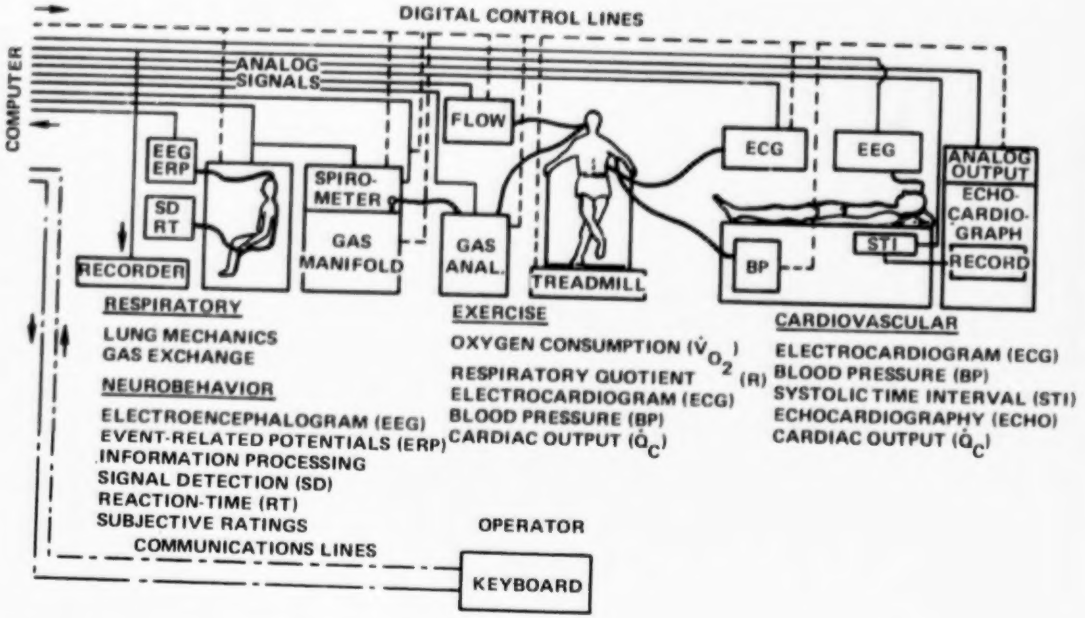
DIGITAL CONTROL LINES
COMPUTER
ANALOG SIGNALS
EEG ERP
SD RT
RECORDER
SPIRO-METER
GAS MANIFOLD
FLOW
GAS ANAL.
TREADMILL
ECG
EEG
BP
STI
ANALOG OUTPUT
ECHO-CARDIO-GRAPH
RECORD
RESPIRATORY
LUNG MECHANICS
GAS EXCHANGE
NEUROBEHAVIOR
ELECTROENCEPHALOGRAM (EEG)
EVENT-RELATED POTENTIALS (ERP)
INFORMATION PROCESSING
SIGNAL DETECTION (SD)
REACTION-TIME (RT)
SUBJECTIVE RATINGS
EXERCISE
OXYGEN CONSUMPTION ($\dot{V}_{O_2}$)
RESPIRATORY QUOTIENT (R)
ELECTROCARDIOGRAM (ECG)
BLOOD PRESSURE (BP)
CARDIAC OUTPUT ($\dot{Q}_C$)
CARDIOVASCULAR
ELECTROCARDIOGRAM (ECG)
BLOOD PRESSURE (BP)
SYSTOLIC TIME INTERVAL (STI)
ECHOCARDIOGRAPHY (ECHO)
CARDIAC OUTPUT ($\dot{Q}_C$)
OPERATOR
KEYBOARD
COMMUNICATIONS LINES

Figure 10-1. Schematic representation of the testing facility and psychophysiological functions to be studied in humans during prolonged exposure to low-level environmental insult. The computerized exposure, monitoring, and physiological data acquisition system will permit precise control of experimental variables. Four subjects can be tested simultaneously in each live-in chamber. (Drawing courtesy of R. W. Stacy, Ph.D.

toxicologic effects of oxidants, acid aerosols, odors, noise, and microwave radiation.

Event-Related Potentials in Human Toxicological Research

Event-related potentials (ERPs) of the brain may be recorded noninvasively from the scalp of man or from electrodes implanted in specific neural structures of animals. Neuroelectric "events" include sensory evoked potentials, association potentials, and motor-related potentials. Sensory evoked potentials are *elicited* by external stimulation of auditory, visual, or somatosensory receptors and reflect the arrival of afferent signals from peripheral receptors. Association potentials reflect a later stage of information processing related to the evaluation of incoming signals which have already been registered. Association potentials presumably index the activity of corticocortical or thalamocortical circuits involved in central decision-making processes. Motor-related potentials reflect cortical processes associated with the preparation for and execution of motor responses, processes which include signals *emitted* from the central nervous system to peripheral effectors.

William Grey Walter (1964), a British neurophysiologist, first described a slow negative shift in electrical activity of the brain related to sensorimotor conditioning in man. This discovery spawned the rapidly growing study of event-related potentials. ERP investigators encompass many disciplines including psychology, psychiatry, neurology, physiology, and pharmacology. To the psychologist, ERPs offer a long-sought quantitative handle on mental processing, an index of activity in the "black box" intervening between stimulus and response. To the behavioral toxicologist, ERPs offer a noninvasive index of chemical insult at the various stages of information processing in the central nervous system. The advantages of ERP techniques in human toxicological research will be reviewed below.

Noninvasive Measure of CNS Function

Ethical considerations preclude the use in human subjects of techniques which require surgical intervention or which pose a threat to safety or health. ERPs provide a noninvasive measure of CNS function which can be used without risk in human research. Some neurotoxins such as industrial solvents produce peripheral rather than central neuropathy (Seppalainen, 1975; Zappoli *et al.*, in press). Traditional electroneuromyographic measures, such as maximal nerve conduction velocity, may be more sensitive than ERPs with substances which produce peripheral neuropathy. On the other hand, peripheral deficits should be reflected in decreased amplitude and/or increased latency of early sensory evoked potential components in the impaired modality. Lead poisoning, for instance, produces marked peripheral neuropathy. Somatosensory evoked potentials (SEP) elicited by stimulation of the damaged afferent pathway should reflect this deficit centrally. Supportive evidence was recently reported by Seppalainen (in press) who found that the SEP was more sensitive than peripheral measures in diagnosing subclinical neuropathy in workers chronically exposed to lead in a storage battery factory.

Sensitivity to Drug Effects

Many substances consumed for pleasure or medicinal purposes may be toxic if used in excess or may produce altered states of consciousness in lesser concentrations. Alcohol is a CNS depressant whose intoxicating and anesthetic qualities have been used and abused for millennia. The tragic consequences of drug abuse and addiction are a direct concern of the clinician. On the other hand, the systematic administration of drugs whose behavioral and neurophysiological mechanisms are known can provide the experimental toxicologist with a useful tool for comparative evaluation of unknown chemical substances. The prospective sensitivity of event-related potentials to neurotoxins can be similarly gauged by examining the effects on ERPs of commonly used psychotropic drugs including stimulants, depressants, and tranquilizers.

Considerable evidence is available on the effects of psychotropic drugs on the contingent negative variation (CNV), a slow negative ERP observed during a signaled interval preparatory to motor response or cognitive decision. In general, CNV amplitude is enhanced by stimulants–amphetamine (Kopell *et al.,* 1974) and caffeine (Ashton *et al.,* 1974)–and decreased by depressants and tranquilizers–barbiturates (Kopell *et al.,* 1974), chlorpromazine (Tecce *et al.,* 1975), carbon monoxide (Groll-Knapp *et al.,* 1972), ethanol (Kopell *et al.,* 1972) and nitrazepam (Ashton *et al.,* 1974). In cases where the behavioral effect of drugs has been paradoxical, CNV changes have consistently mirrored behavioral observations. That is, when a given drug enhanced performance in some subjects but depressed performance in others, CNV amplitude varied in the same direction as performance. Paradoxical effects have been reported with d-amphetamine (Tecce and Cole, 1974) and nicotine (Ashton *et al.,* 1973). This evidence suggests that the CNV should provide a useful index of central-acting neurotoxic drug effects.

Convergent and Discriminant Validity

ERP techniques offer the advantage of simultaneous behavioral and electrophysiological measurement of toxicant effect. That is, ERPs are usually recorded during performance of sensorimotor conditioning, signal detection, or vigilance tasks which yield concomitant behavioral measures. Multiple measures lend convergent validity to results when two or more measures vary consistently or correlate highly. Groll-Knapp *et al.* (1972) demonstrated convergent validation of a vigilance decrement during low-level carbon monoxide exposure. Signal detection and the amplitude of a slow negative brain potential both varied inversely with CO concentration.

On the other hand, it would be redundant to collect both types of information if behavioral and electrophysiological measures always yielded convergent results. The data would be more informative if the measures were orthogonal, i.e., indexed independent dimensions. The discriminant validity of these measures is the degree to which ERP and behavioral parameters reflect orthogonal dimensions. ERP and behavioral measures frequently do not covary. Reaction-time, a traditional behavioral index of central processing time, can be easily dissociated from the CNV observed during the reaction-time fore-period

Rebert and Tecce, 1973). Similarly, the observed electrophysiological effects of toxicant exposure might be orthogonal to behavior, i.e., ERP changes might be observed in the absence of a behavioral deficit. Winneke and Kaska (1974), for instance, observed a decrease in amplitude of the AEP without impairment of vigilance performance in subjects exposed to trichloroethylene. The irony of the Winneke and Kaska finding is that the functional significance of the ERP deficit is difficult to evaluate in the absence of convergent behavioral validation.

Localization of Neurotoxic Effects

Although the precise neuroanatomical substrate of most event-related potentials is presently unknown, it is generally assumed that the temporal sequence of early components in sensory evoked potentials reflects the serial transmission of signals along afferent pathways from distal (brainstem) to proximal (cortical) structures. Evidence is rapidly accumulating that later components reflect successive stages of information processing at the cortical level. Picton *et al.* (1974), for instance, have identified fifteen distinct components in the auditory evoked potential as shown in Figure 10-2. They attribute early components (within 8 msec of stimulus onset) to activation of cochlea and brainstem auditory nuclei, middle components (8-50 msec) to activation of the auditory thalamus and cortex, and late components (50-300 msec) to diffuse activation of frontal cortex.

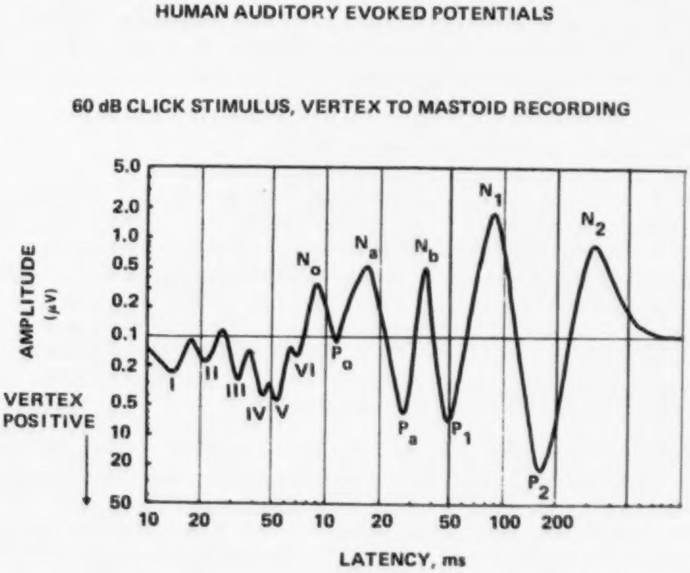

Figure 10-2. Diagram of auditory evoked potential components plotted on logarithmic scales (from Picton *et al.*, 1974 courtesy of Elsevier Scientific Publishing Company).

Table 10-1 summarizes contemporary evidence on the neuroanatomical origin of AEP components. Note that components with latencies greater than 20 msec are presumed to be of cortical origin. The topographic recording and mapping of middle and late components permit at least a crude localization of the source of these components within the cortical mantle. As the neuroanatomic origins of ERP components are elaborated, ERP techniques will become an increasingly important tool for localizing the site of neurotoxic action.

Table 10-1. PROBABLE NEUROANATOMICAL SOURCE OF AUDITORY EVOKED POTENTIAL COMPONENTS[a]

Component	Latency, msec	Probable source	Reference
I	<2	Auditory nerve fiber	Jewett & Williston, 1971
II – IV	2–5	Cochlea & superior olivary nuclei	Galambos *et al.*, 1959
V, VI	6–7	Lateral lemniscus or inferior colliculus	Webster, 1971 Kitahata *et al.*, 1969
N_o, P_o, N_a	8–20	Medial geniculate & non-specific thalamic nuclei	Picton *et al.*, 1974
P_a, N_b	21–49	Anterior & posterior association cortex	Ibid.
P_1, N_1 P_2, N_2	50–300	Widespread activation of frontal cortex	Goff *et al.*, 1969 Kooi *et al.*, 1971 Hardin & Castellucci, 1970

[a] Based on T. W. Picton *et al.*, 1974.

Neurotoxic Effect on Information Processing

Neurotoxic effects on complex integrative (or cognitive) functions of the brain have been inferred from behavioral measures in the past (e.g., Beard and Grandstaff, 1975). Event-related potentials offer a more direct, remarkably sensitive index of cognitive processing in the central nervous system. Theories of signal detection (Green and Swets, 1966) and information processing (Broadbent 1958, 1971; Treisman, 1969) have provided the conceptual framework for investigating the relationship of ERP components and cognitive processes in man. Tueting (in press) has critically reviewed this rapidly-expanding field of research. Hillyard *et al.* (1973), Picton *et al.* (1974), Picton and Hillyard (1974), and Schwent and Hillyard (1975) have intensively studied the functional significance of auditory ERPs. Evidence from these studies suggests that components with latencies in the 60-120 msec range reflect attentional processes related to stimulus selection or "stimulus set," while later components (160-500 msec) reflect decisional processes of response selection or "response set."

The amplitude of a large positive polysensory* wave, peaking 200-400 msec after stimulus onset (designated P300), varies inversely with the probability of stimulus occurrence (Tueting *et al.*, 1971). Probability of occurrence is also a theoretical definition of information content. The P300 has been associated in other studies with decision-making (Davis, 1964), information delivery (Sutton *et al.*, 1967), and response inhibition (Papakostopoulos and Crow, 1976). In brief, considerable evidence has accumulated that ERPs reflect multiple parameters of information processing in man. ERPs should, therefore, provide an excellent, noninvasive electrophysiological index of neurotoxic insult on cognitive function.

The Neurobehavioral Effects of Low Frequency Noise in Humans: An Application of ERP Methods

The following study was designed to assess the effects of low frequency random noise (11.5-325 Hz) at moderate intensity (80 db) on ERPs and behavior. The results have been reported in detail elsewhere (Benignus *et al.*, 1975 and Otto *et al.*, in press). Selective aspects will be elaborated here to illustrate basic principles of ERP methodology.

Rationale

Although discussion in this chapter has been oriented toward chemical substances, the behavioral toxicologist is also concerned with physical insults to the brain including noise and electromagnetic radiations. Noise is a staple in urban man's diet of carbon monoxide, oxidants, acid aerosols, and heavy metal particulates. Noise is an omnipresent stressor capable of severely disrupting behavior or physically damaging the auditory system at high intensities.

Many researchers routinely use white noise to mask extraneous laboratory sounds during testing. What is the effect of masking noise on human performance? That question underlies the following experiment in which the level of noise was not perceived as particularly irritating or distracting. The synergistic effects of noise and noxious chemical substances which frequently occur together in experimental as well as real-life situations are also or empirical interest.

Vigilance tests have been widely used in studies of stress (cf. Broadbent, 1971). The vigilance decrement—a measure of target signals missed over time—appears to be extremely sensitive to both physical and chemical stressors. A continuous performance vigilance test designed by Rosvold *et al.* (1956) to minimize fluctuations in alertness was used as the behavioral context. Mirsky and Rosvold (1960) found this test to be sensitive to psychoactive drug effects. McCallum (1975) demonstrated its utility in recording event-related potentials.

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*That is, the observed effects are independent of sensory modality.

Methods

Subjects

Twenty-seven male university students, aged 18-31, were paid to participate. All subjects were carefully screened to determine that hearing (Bekesy audiometer), personality (Minnesota Multiphasic Personality Inventory), and health (physical examination) were within normal limits.

Continuous Performance Test (CPT)

Single-digit numerals were displayed at one-second intervals in pseudorandom series. Subjects were instructed to press a button whenever a sequence of three consecutive even digits or three consecutive odd digits (a three-string) was observed. Digits were presented in nine 12.5-minute series, each containing about 111 target three-strings. Three-minute rest breaks were interspersed between work periods.

The CPT imposes greater information processing demands than classical vigilance or signal detection tasks. Stimuli in a standard vigilance task require a simple binary decision—target or nontarget. The numeric monitoring task described here includes a short-term memory component. In order to identify a target correctly, subjects must recall the previous two stimuli. Target identification also requires more than simple physical matching of stimuli. Each numeral must be coded as "even" or "odd" and this "polarity" dimension must be held in short-term memory. No single stimulus or simple template match provides the solution.

More complex questions can be addressed with a three-string sequential dependency than with a binary-choice problem. For instance, the proportion of target and neutral stimuli cannot be varied independently in a binary vigilance problem. One factor is always the converse of the other. In the ternary problem, however, the value of one factor does not uniquely define the values of the other two factors. Benignus *et al.* (1976) have exploited this feature to reassess the effect of signal rate on vigilance performance.

If the reader is momentarily experiencing a decline in vigilance, the discussion bears out Occam's razor: parsimony is the essence of good experimental design! The high level information-processing capabilities of *Homo sapiens* may tempt the behavioral toxicologist to needless levels of complexity. The theoretical challenge of the CPT notwithstanding, a simple binary-choice vigilance task would be better suited for interspecies comparison of toxicological effects!

Experimental Design

Subjects completed orientation, stabilization, and testing sessions on three consecutive days. The first day consisted of an abbreviated training session to familiarize subjects with the task and laboratory setting. On the second day electrophysiological recording procedures were introduced and the subjects were trained to fixate on a visual target to minimize eye movements.*

Subjects also completed nine numeric-monitoring series during which performance reached asymptote. The same procedure was repeated on the third day. Analyses are based on stabilized performance recorded on the final day.

The output of a random-noise generator was bandpass-filtered to obtain a 91-325 Hz noise spectrum and was then tape-recorded at 15 inches per second (IPS). A second noise spectrum (11.4-41 Hz) was obtained by playing the tape back at 1 7/8 IPS. These bandwiths will be designated hereafter as upper (U) and lower (L). The noise was presented free-field through a loudspeaker placed about 1.9 m in front of subjects.**

Noise and no-noise control (C) conditions were alternated during successive work periods on days 2 and 3 as follows: CUCLCLCUC or CLCUCUCLC. Noise exposure schedules were counterbalanced across subjects with each one exposed to a different schedule on succeeding days. Subjects were instructed to ignore the noise.

An alternating repeated-measures design is useful only when the effects of a stressor are presumed to be rapidly reversible. It is *not* practical with chemical compounds which accumulate in body tissue during exposure. In any repeated-measures study, sufficient time for a complete return to physiological baseline must be allowed between successive exposures. This may be a matter of hours, days, or months, depending on the biological half-life of the test compound. Liposoluble substances such as DDT and dieldrin, for instance, accumulate in the fatty tissues of mammals (cf. Walker, 1974) where residues may persist for years. Independent groups are more practical (or mandatory) with persistent substances such as heavy metals or organochlorine insecticides.

Electrophysiological Procedures

The recording of electrophysiological parameters is a relatively simple procedure but requires sophisticated electronic equipment. Figure 10-3 is a schematic diagram of the basic components required to record psychophysiological parameters such as the electroencephalogram (EEG), electro-oculogram (EOG), electromyogram (EMG), and electrodermal response (EDR). Excellent descriptions of recording techniques are available elsewhere (e.g., Venables and Martin, 1967; Greenfield and Sternbach, 1972; Thompson and Patterson, 1974). Only a brief overview is provided here.

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*Eye movement artifact can severely contaminate ERP recordings if subjects do not fixate on a visual target. Normal adults can be easily trained to fixate. Eye movement control poses a serious problem in young children and abnormal adult populations (i.e., psychotics or sociopaths) where subjects are unable or refuse to fixate. Hillyard (1974) describes techniques that can be used to compensate for eye movement atrifact under these circumstances.

**See Benignus *et al.* (1975) for technical details of noise generation.

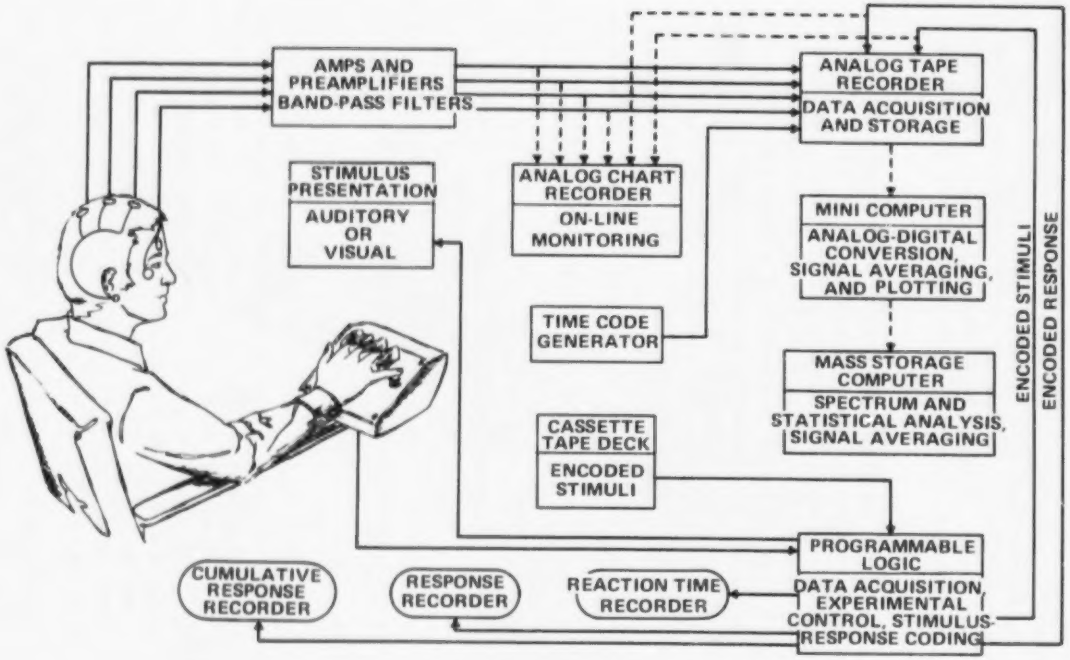
AMPS AND PREAMPLIFIERS BAND-PASS FILTERS
ANALOG TAPE RECORDER
DATA ACQUISITION AND STORAGE
STIMULUS PRESENTATION
AUDITORY OR VISUAL
ANALOG CHART RECORDER
ON-LINE MONITORING
MINI COMPUTER
ANALOG-DIGITAL CONVERSION, SIGNAL AVERAGING, AND PLOTTING
TIME CODE GENERATOR
MASS STORAGE COMPUTER
SPECTRUM AND STATISTICAL ANALYSIS, SIGNAL AVERAGING
CASSETTE TAPE DECK
ENCODED STIMULI
ENCODED STIMULI
ENCODED RESPONSE
PROGRAMMABLE LOGIC
DATA ACQUISITION, EXPERIMENTAL CONTROL, STIMULUS-RESPONSE CODING
CUMULATIVE RESPONSE RECORDER
RESPONSE RECORDER
REACTION TIME RECORDER

Figure 10-3. Diagram of basic equipment needed to record event-related potentials of the brain and other psychophysiological parameters of human performance.

Nonpolarizing electrodes are attached to the scalp and appropriate skin areas with commercially-available pastes or adhesive collars for short-term exposures. Collodion provides a more stable bond for longer exposures. The International 10-20 Electrode System (Jasper, 1958) is generally used as a reference for EEG electrode placement to standardize procedures among laboratories. It is mandatory in ERP research to record vertical EOG in order to identify records containing eye movement artifact. If movement-related potentials are to be measured, it is also important to record the EMG of responding muscle groups for comparision of central waveforms and the temporal pattern of distal motor unit discharge.

In the present study, nonpolarizable silver-silver chloride Beckman biopotential pellet electrodes were placed at the vertex (C_z) and over the right (P_3) and left (P_4) mid-parietal association areas. Electrodes were also placed above the inner canthus and below the outer canthus of the left eye to monitor and record eye movements. Scalp electrodes were referred to a linked pair of electrodes clipped to the earlobes. The earlobes are considered to be electrically neutral so that electrical potentials recorded differentially between scalp electrodes and linked ears can be assumed to reflect activity at the scalp recording site. Parietal recordings were made to investigate the effects of low-frequency noise on the coherence of spontaneous EEG rhythms in the left and right hemispheres. This report will be limited to ERP components observed at the vertex.

The amplitude range of spontaneous EEG rhythms in normal adults is 2-200 μV (see Table 10-2). It is necessary, therefore, to amplify the spontaneous EEG by as much as 10^9 to reach the minimum 1 V peak-to-peak deflection required by IRIG, *Telemetry Standards (1969). Amplified signals are then recorded on FM tape or channelled directly to a computer for analysis. If laboratory facilities permit, electrophysiological data should be processed on-line for immediate availability of results. Frequently, on-line computer access is not practical, and the data is recorded on analog tape for off-line processing.

Signal Averaging

How are EEG signals processed? In signal averaging the first step is to convert the continuous EEG record to a series of discrete voltage samples—a process called analog-to-digital conversion. The digitizing rate determines the minimum frequency of ERP components which can be resolved. That is, the digitizing rate should be at least twice the frequency of the fastest component to be measured. Since most ERP components are slower than 50 Hz, a digitizing rate of 100 Hz is adequate in most cases. Analysis of brainstem evoked potentials which occur within the first 10 msec following stimulus onset, however, requires a digitizing rate of at least 2000 Hz.

The second step is signal averaging in which digitized arrays of data from successive trials time-locked to a repetitive event are summed and divided by the number of trials. The triggering event is usually an external stimulus but

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*Inter-Range Instrumentation Group.

may also be a response mechanogram, EMG burst, or any discriminable physiological signal.

Table 10-2. CHARACTERISTICS OF SPONTANEOUS EEG RHYTHMS IN NORMAL HUMAN ADULTS[a]

Type	Frequency (c/sec)	Amplitude (μV)	Level of consciousness
Delta	0.5–4	20–200	Slow wave sleep
Theta	5–7	5–100	Slow wave sleep
Alpha	8–12	5–100	Awake, relaxed
Beta	18–30	2–20	Awake, concentrating or rapid eye movement (REM) sleep

[a] Adapted from Lindsley and Wicke (1974, p 26).

Why is signal averaging necessary? Scalp-recorded ERP components are frequently submerged in a turbulent sea of spontaneous EEG rhythms. For instance, brainstem evoked potentials are 1 μV or less, sensory evoked potentials are 5-15 μV, and association potentials are 5-25 μV in amplitude. Spontaneous EEG rhythms range from 2-200 μV in normal adults as shown in Table 10-2. Signal averaging is an effective method of increasing the ERP signal-to-EEG noise ratio.

Signal averaging assumes that the temporal characteristics of ERPs are invariant with respect to the triggering event. That is, the signal is assumed to be stationary in time. In statistical terms, the mean and variance of the signal should be constant within the averaging epoch and across successive trials. The validity of this assumption is dependent on the length of time required to collect trials for the average. Unfortunately, *Homo sapiens* has little respect for statistical theory and tends to be notoriously variable. ERP changes due to alterations in arousal, attention, fatigue, habituation, learning, or other dynamic neural processes are obscured by signal averaging. This problem is minimized by using as few trials as possible in the construction of averages.

How many trials should be included in a given signal average? As a rule of thumb, the amplitude ratio of spontaneous EEG to ERP is proportional to the square root of the number (N) of trials ($EEG/ERP = \sqrt{N}$). Table 10-3 provides a simple guide to determine N. ERP and EEG values represent peak-to-peak measurements.

Rigorous treatment of the theory, methods, and problems of signal averaging are beyond the scope of this review. Procedures and problems specific to ERP research are discussed in depth in Donchin and Lindsley (1969), McCallum and Knott (1973, 1976) and Thompson and Patterson (1974). Vaughan (1974) provides a particularly lucid account.

Table 10-3. **MINIMUM NUMBER OF TRIALS REQUIRED FOR SIGNAL AVERAGING AS A FUNCTION OF ESTIMATED ERP AMPLITUDE AND AVERAGE AMPLITUDE OF SPONTANEOUS EEG**

Estimated ERP amplitude (μV)	Number of trials/average			
	EEG Amplitude (μV) 25	50	100	Type of ERP
0.5	2,500	10,000	40,000	Brain stem evoked potentials
1.0	625	2,500	10,000	
2.0	156	625	2,500	Sensory evoked potentials
5.0	25	100	400	Association potentials
10.0	6	25	100	
25.0	1	4	16	

Results

Changes in numeric-monitoring efficiency and ERP amplitude were observed during exposure to low-frequency noise. Figure 10-4 illustrates the behavioral effects.* In brief, a vigilance decrement across time was observed in all conditions. Subjects missed more target strings during both upper ($p < 0.005$) and lower band ($p < 0.039$) series than during control series. The difference in performance between upper and lower noise series was not significant.

Complex relationships between noise conditions, performance, and ERP components were also observed. ERP averages were constructed separately for hit, miss, and correct rejection trials. Due to excessive EOG artifact in some subjects, only 21 subjects were used in ERP analyses. Large intersubject variance in performance further reduced the number of usable subjects in hit and miss analysis. To illustrate the range of performance, subjects were rank-ordered according to the total number of misses and then divided into three equal groups. The average number of misses per series for subjects in high, medium, and low performance groups is shown in Table 10-4. Subjects in the high performance group missed so few trials that meaningful averages of "miss" data could not be constructed. The converse problem was encountered to a lesser extent with "hit" data in the low performance group.

Hit Analysis

Hits were defined as three-string sequences which subjects correctly detected. Sufficient hit trials were obtained to construct averages for every control and noise series in 17 subjects. A typical averaged waveform recorded at the vertex is shown in Figure 10-5 to illustrate ERP measurement procedures.

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*See Benignus *et al.*, (1975) for technical details of the statistical analysis of behavioral results.

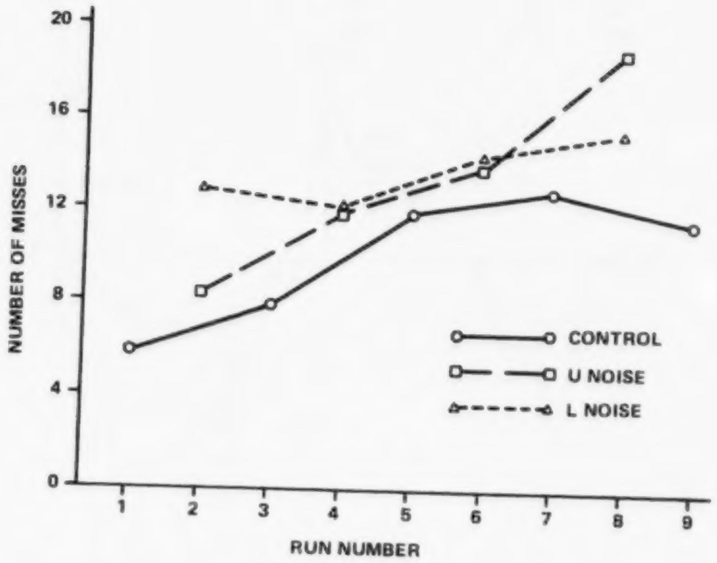

Figure 10-4. Number of misses across time for control, upper and lower noise bands. (Reprinted with permission of publisher from : Benignus, V. A., Otto, D. A., and Knelson, J. H. (1975). Effect of low-frequency random noises on performance of a numeric monitoring task. *Perceptual and Motor Skills* 40, 231-239.

Table 10-4. MISS RATES DURING CONTROL VS. NOISE CONDITIONS FOR HIGH, MEDIUM & LOW PERFORMANCE GROUP

Performance level	Average misses/series	
	Control	Noise[a]
High (N = 7)	2.2	3.0
Medium (N = 7)	7.0	8.4
Low (N = 7)	26.9	38.8
± 1.96 x SE[b]	±2.69	±4.68

[a] Upper and lower bands combined.

[b] 95% confidence Interval = mean ±. 1.96 × standard error of mean.

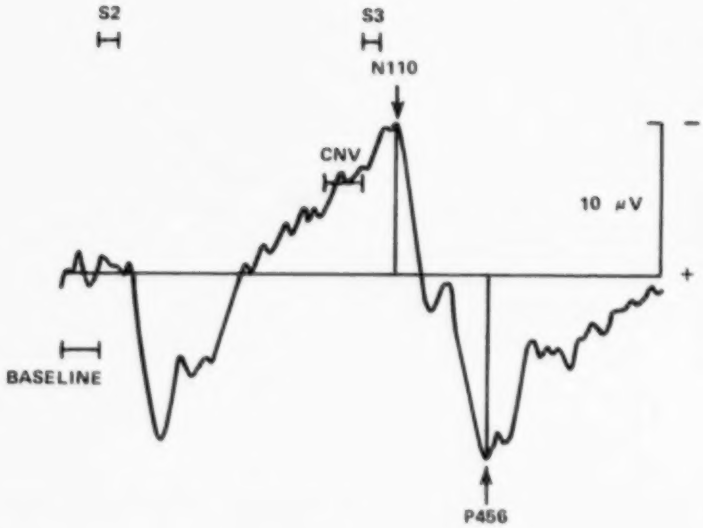

Figure 10-5. Measurement procedures for components of a typical event-related potential waveform. S2 and S3 designated the intervals during which the second and third digits of 3-string sequences were displayed. Contingent negative variation (CNV) was measured as the average voltage during the specified interval relative to the pre-S2 baseline. Baseline-to-peak measurements were made of negative (N110) and positive (P456) components following S3. Timebase: one sec interstimulus interval (S2 onset - S3 onset).

Three ERP components designated CNV, N110, and P456 were measured. The CNV was computed as the mean amplitude of a 256 msec epoch preceding the third digit (S3) relative to a 256 msec baseline epoch preceding the second digit (S2). N110 and P456 were measured from the peaks in each individual average to the same baseline. The convention used to label N110 and P456 components specifies the polarity—negative (N) and positive (P)—and the average peak latency from stimulus (S3) onset expressed in msec.

Table 10-5 shows ERP amplitudes during hit trials in control, upper, and lower band noise series. Small decrements in the amplitude of each measure were observed in noise compared to control conditions. In order to reduce the dimensionality of the data (and thereby increase the power of statistical tests), control means were subtracted from upper and lower band means for each subject. The effect of noise on ERPs was tested against the null hypothesis that none of the differential ERP scores differed from zero. A single-factor multivariate test with Wilks-Lambda criterion converted to an F ratio by Rao's procedure (Cooley and Lohnes, 1971) was used to reject the overall null hypothesis ($F_{6,11} = 6.40$, $p < 0.004$).

Table 10-5. HIT TRIALS: ERP AMPLITUDE (μV) AS A FUNCTION OF NOISE EXPOSURE

	CNV	N110	P456
Control	−3.9	−5.7	13.1
Upper Band	−3.8	−4.7	12.0
Lower Band	−3.1	−4.4	12.7
± 1.96 × SE[a]	0.43	0.77	0.98

[a] 95% confidence interval = mean ± 1.96 × standard error of mean.

Table 10-6 provides the results of *post hoc* univariate tests for each component. N110 amplitude decreased significantly during upper and lower band noise exposure; CNV decreased during lower band noise only; and P456 was not significantly affected by noise. In order to preclude the possiblity that the N110 measure was confounded by the slow negative shift preceding S3, CNV values were subtracted from N110 values of each subject prior to statistical testing.

Table 10-6. SUMMARY OF UNIVARIATE F TESTS TO EVALUATE EFFECTS OF NOISE ON ERP COMPONENTS IN HIT AVERAGES

Null hypothesis	Mean Sq	F(1,16)	P
(Lower Band CNV) = 0	11.35	8.53	⟨0.010
(Upper-Band CNV) = 0	0.41	0.31	⟨0.585
(Lower-Band N110) = 0	2.97	4.35	⟨0.053
(Upper Band N110) = 0	10.20	10.93	⟨0.004
(Lower-Band P456) = 0	3.17	0.67	⟨0.426
(Upper Band P456) = 0	13.34	1.54	⟨0.233

Correct Rejection (CR) Analysis

CR trails were defined as two-strings to which subjects did not respond. The "no-response" contingency, it should be noted, provided no indication of whether or not the subjects processed the digits. That is, the absence of response may have reflected either correct identification of a two-string sequence or a lapse in attention. Twenty-one subjects were used in this analysis. CNV, N110, and P456 components were measured in CR averages in the same manner as in hit averages. None of these measures, however, significantly discriminated between noise and control conditions. The effects on ERPs of performance was also examined. Table 10-7 shows the amplitude of CNV, N110 and P456 averaged across control and noise conditions for high, medium, and low performance groups. Although decrements in all ERP components were observed in the low performance group compared to medium and high performance subjects, only P456 differences approach significance.

Table 10-7. CORRECT REJECTION TRIALS: ERP AMPLITUDE (μV) AS A FUNCTION OF MONITORING PERFORMANCE

Performance Level	CNV	N110	P456[a]
High (N = 7)	−4.2	−6.7	12.0
Medium (N = 7)	−4.1	−5.8	10.5
Low (N = 7)	−3.2	−5.2	6.7
±1.96 x SE	±0.63	±0.74	±1.22

[a] $F(2,18) = 3.098$, $p < 0.07$.

Miss Analysis

In view of the ambiguous nature of two-string events, a direct comparison of hit and miss trials was undertaken to further assess the effects of performance on ERPs. Miss trials were precisely defined as three-string sequences which subjects failed to detect. In previous analyses, *averages* for each successive control and noise series were constructed *without saving single trials*. This procedure could not be followed in this analysis since miss trials had to be pooled across multiple series in order to accumulate sufficient trials for averaging. Even pooling across series did not yield meaningful miss averages in many high performance subjects. Therefore, the miss analysis was restricted to the low performance group. Hit and miss trials of these subjects were redigitized and saved individually. Successive averages of 24 trials were then constructed for hits and misses, irrespective of control and noise conditions.

Hit and miss trials averaged across all conditions and subjects in the low performance group are superimposed in Figure 10-6. Note the diminution of CNV, N110, and the absence of P456 in the average of miss trials. Mean amplitudes of ERP components in hit and miss averages are shown in Table 10-8. A multivariate test was used to evaluate the null hypothesis of no difference between hit and miss ERPs ($F_{12,25} = 13.665$, $p < 0.001$).* Univariate F-tests were significant for all hit-miss ERP comparisons as indicated in Table 10-8.

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*Data obtained from left and right parietal electrodes, as well as the vertex, were included in this analysis. Consistent results were observed at all recording sites.

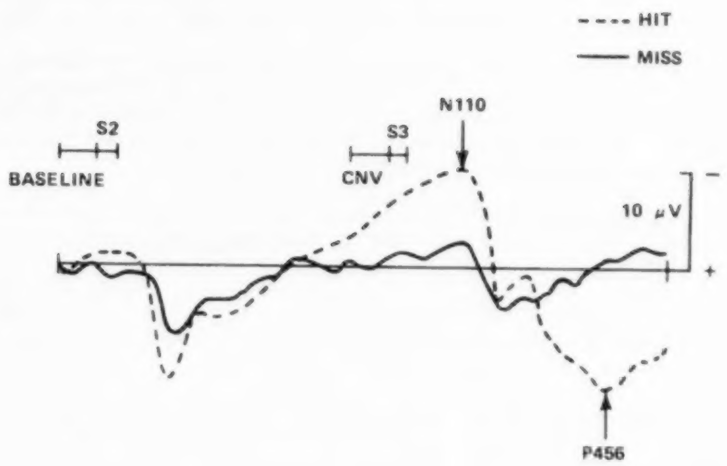

Figure 10-6. Summary waveforms of hit and miss trials averaged across all noise and control series of 7 low performance subjects. Note the attenuation of CNV, N110 and absence of P456 during miss-trials. Abbreviations as in Figure 10-5.

Table 10-8. LOW PERFORMANCE SUBJECTS (N = 7): ERP AMPLITUDE (μV) AS A FUNCTION OF HITS VS. MISSES

	CNV	N110	P456
Hits	−4.3	−10.5	12.2
Misses	0.5	−0.9	1.8
F(1,36)	7.197	26.956	15.243
P(	0.011	0.001	0.001

Discussion

Results of this study indicate that low-frequency continuous noise at moderate intensity affects vigilance performance and at least one ERP component. What is the functional significance of these findings? Interpretation of the behavioral results is unequivocal: vigilance performance is slightly impaired. Extrapolation from a monotonous numeric-monitoring task in a small isolated chamber to real-life requires only a minimal leap in imagination. An individual driving cross-country on a superhighway may experience analogous conditions of monotony and social isolation. Engine hum, tires, and air-conditioning may generate low-frequency noise of comparable intensity. High-speed driving certainly demands continuous vigilance to visual signals. The consequence of even a slight impairment in driving vigilance needs no elaboration.

The effect of noise on the N110 component was observed in an analysis of "hit" trials. The interpretation of the N110-noise relationship is weakened by the lack of effect in a subsequent analysis of "correct rejection" trials. The ambiguous behavioral significance of CR events, however, renders the interpretation of associated ERPs difficult.

The final comparison of "hit" versus "miss" averages in the low performance group illustrates the sensitivity of ERPs to behavioral contingencies. CNV and N110 components were sharply reduced, and the P456 component was completely absent in miss trials. Are these differences merely a reflection of the presence or absence of an overt behavioral response (i.e., motor artifacts)? CR averages rule out this possibility. CNV, N110, and P456 components were clearly present in CR averages.

What is the significance of the change in N110 amplitude observed during exposure to low-frequency noise? The neurophysiological mechanism underlying the N110 is unknown. Cautious inferences may be drawn from the associated behavioral decrement. Convergent decrements in observed behavioral and electrophysiological measures could reflect an impairment in either selective or general attention.

Available evidence does not support a general attentional or arousal decrement hypothesis. First, a decrease in P456 amplitude would be expected if the effect were nonspecific. Second, there is little reason to suspect that N110 amplitude would *decrease* as a function of arousal level. Picton *et al.* (1974) did not observe any change in auditory ERP components earlier than 250 msec during the transition from wakefulness to sleep. In fact, the predominant change in auditory ERPs as subjects fell asleep was a dramatic *increase* in the amplitude of a negative component peaking at 290 msec. Results of a spectrum analysis of the present data (Benignus *et al.*, in preparation) also failed to support the arousal hypothesis. If low-frequency noise induced drowsiness a decrease in beta and alpha frequencies accompanied by an increase in theta and delta frequencies would be expected. No difference in the frequency distribution of spontaneous EEG during control versus noise series was observed.

What evidence can be marshalled in support of a selective attentional hypothesis? Schechter and Buchsbaum (1973) observed consistent changes in P110-N140 (peak-to-peak) amplitudes of auditory and visual evoked potentials in a series of tasks with graded attentional demands. The P100-N140 component was smallest in a "distraction" condition. Schwent and Hillyard (1975) provide convincing evidence of intramodal selective attentional effects in an auditory N1 component analagous to N110. They observed a marked enhancement of N1 in "attend" compared to "ignore" conditions.

These studies indicate that a polysensory negative ERP component with a latency of about 100 msec may reflect selective attentional processes. The convergent behavioral and ERP results of the present study suggest, therefore, that low-frequency noise selectively interferes with the ability of subjects to "tune in" or concentrate on incoming sensory signals. That is, low-frequency noise at moderate intensity may impair selective attention but does not seem to affect general arousal in a vigilance-like task.

Concluding Comment

Event-related potentials provide an intriguing, noninvasive window on sensorimotor and cognitive functions in man. There are optimistic signs that research during the next decade will resolve many of the current mysteries concerning the neuroanatomical mechanisms and functional significance of ERPs. Considerable progress has been made in the study of the brain stem evoked potentials, the P300, and the CNV through the close alliance of behavioral and electrophysiological testing procedures.

Do ERP techniques currently offer a useful tool to the behavioral toxicologist? This article has presented arguments that ERPs provide a noninvasive index of neurotoxic effect on CNS function and information processing. The conviction of this author is not shared universally, however, and a dash of skepticism is appropriate to complete the story.

S. S. Stevens, the late Harvard psychophysicist eloquently summarized the negative view (1971, p. 502):

> An electrode on the skull may or may not be able to reflect
> the operation of the sensory transducer in a meaningful way.
> The full answer to that question remains to be discovered... .
>
> Granting all the difficulties, I would like to believe that
> the knowledge that can be gleaned about sensory systems by
> studying evoked potentials is greater than zero.

H. E. Stokinger, Chairman of the Threshold Limits Committee at the National Institute of Occupational Safety and Health, bluntly challenges the utility of ERP data in environmental toxicology: "Changes in the visually evoked response (VER) are presently not sufficiently well understood in over-all physiological terms to serve as basic criteria for air standards" (1974, p. 20).

Stokinger's position is questionable on theoretical grounds because it assumes that evidence of physiological effects requires knowledge of causal mechanisms. In medicine, drugs known to relieve specific symptoms are frequently administered without precise knowledge of physiological mechanisms. Amphetamines, for instance, may be used to reduce hyperactivity, although the mechanism underlying this paradoxical effect is not understood. Behavioral observations are not admissible by Stokinger's logic either, since the physiological mechanisms underlying behavioral decrements are seldom known. The functional significance of behavioral observations, however, is generally self-evident, whereas the functional significance of ERP changes *per se* may be ambiguous. In cases where neither the physiological nor the behavioral significance of an ERP change can be established, Stokinger's position is difficult to refute.

ERP investigators are vigorously amassing evidence to dispel this skepticism. ERP techniques have been applied with modest success to a variety of clinical problems in audiometry (Davis, 1976) neurology (Starr and Achor, 1975; Low *et al.*, 1976), psychiatry (Shagass 1972; Timsett *et al.*, 1973), and developmental disorders (Karrer, 1976). The evidence reviewed in this chapter suggests that ERP techniques can provide a useful research tool in behavioral toxicology as well.

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